A. INGREDIENT NAME:

DIAMINOPYRIDINE (3,4-)

B. Chemical Name:

3,4-Pyridinediamine

C. Common Name:

3,4-DAP, $C_5H_7N_3$

D. Chemical grade or description of the strength, quality, and purity of the ingredient:

98+%

E. Information about how the ingredient is supplied:

Pale brown crystalline powder

F. Information about recognition of the substance in foreign pharmacopeias:

G. Bibliography of available safety and efficacy data including peer reviewed medical literature:

McEvoy, K. M. 4-Diaminopyridine in the treatment of Lambert-Eaton myasthenic syndrome. *N Engl J Med,* 1989; 321: 1567-1571.

Russell, J. W. Treatment of stable chronic demyelinating polyneuropathy with 3,4-diaminopyridine. *Mayo Clin Proc,* 1995; 70: 532-539.

Newsom-Davis, J. Myasthenia gravis and the Lambert-Eaton myasthenic syndrome. *Prescribers' J,* 1993; 33: 205-212.

McEvoy, K. M. Clinical evaluations in myasthenic syndromes. *N Engl J Med,* 1989; 321: 1567.

Bever, C.T., Anderson, P. A., and Leslie, J. Treatment with oral 3,4 diaminopyridine improves leg strength in multiple sclerois patients: results of a randomized, double-blind, placebo-controlled, crossover trial. *Neurology,* 1996; 47(6): 1457-1462.

Oh, S. J., Kim, D.S., and Head, T. C. 3,4-diaminiopyridine, which is not readily available in the United States, is recommended as the preferred drug for LEMS. *Muscle Nerve,* 1997; 20(9): 1146-1152.

Anlar, B., Varli, K., and Ozdirim, E. 3,4-diaminopyridine in childhood myasthenia: double-blind, placebo-controlled trial. *J Child Neurol,* 1996; 11(6): 458-461.

Aisen, M. L., Sevilla, D., and Edelstein, L. A double-blind placebo-controlled study of 3,4-diaminopyridine in amytrophic lateral sclerosis patients on a rehabilitation unit. *J Neurol Sci,* 1996; 138(1-2): 93-96.

H. Information about dosage forms used:

Orally

I. Information about strength:

10-20mg; three to four daily

J. Information about route of administration:

Orally

K. Stability data:

Melts at about 218-220° with decomposition
Incompatibilities: Strong acid, Strong oxidizing agents

L. Formulations:

M. Miscellaneous Information:

CERTIFICATE OF ANALYSIS

30-2569
55200

D

PRODUCT NO: **1824**

PRODUCT: **3,4-Diaminopyridine**

We hereby certify that batch **03630** of the above product has been tested with the following results:

Appearance:	Pale brown crystalline powder E	
Melting Point:	Darkens 213°C	
Elemental Analysis:	**Found(%)**	**Theory(%)**
Nitrogen:	38.53	38.50
Carbon:	54.79	55.03
Hydrogen:	6.49	6.47

Date of Analysis: **9 July 1991**

Signed: [signature] **30 December 1997**

Quality Control Manager

12/97

✓

QUALITY CONTROL REPORT

CHEMICAL NAME.: DIAMINOPYRIDINE (3,4)

MANUFACTURE LOT NO.: 3630

PHYSICAL TEST

SPECIFICATION TEST STANDARD.: USP__/BP__/MERCK__/NF__/MART.__/CO.SPECS.__.

1) DESCRIPTION.:
PALE YELLOW TO YELLOW CRYSTALLINE POWDER; SLIGHT ODOR.

2) SOLUBILITY.:
SOLUBLE IN HOT WATER; SPARINGLY SOLUBLE IN ALCOHOL; SOLUBLE IN HOT ALCOHOL.

3) MELTING POINT.:
MELTS AT ABOUT 218-220 degree WITH DECOMPOSITION.

4) SPECIFIC GRAVITY.:

5) IDENTIFICATION.:

PASSES.: _____ **FAILS.:** _____

COMMENTS.: ABOVE TEST IS CARRIED OUT BY VISUAL OBSERVATION DUE TO LESS AMOUNT SAMPLE. CHEMICAL LABEL NAME ON BOTTLE - 3,4-DIAMINOPYRIDINE, 98+ %.

ANALYST SIGNATURE.: _____ **DATE.:** _____

PREPACK TEST.: _____ **DATE.:** _____ **INITIAL.:** _____

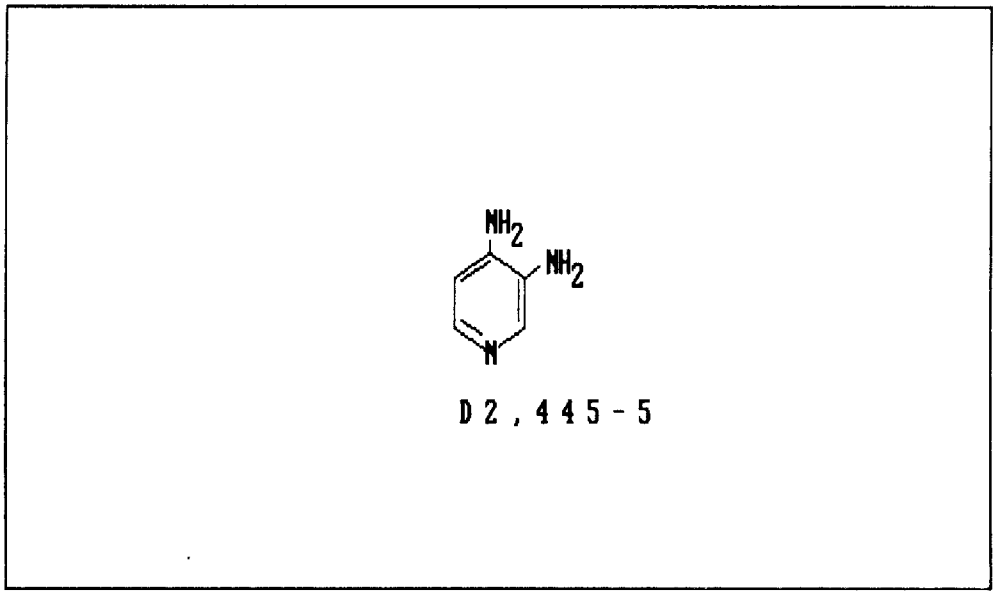
RETEST.: _____ **DATE.:** _____ **INITIAL.:** _____

MATERIAL SAFETY DATA SHEET

Sigma-Aldrich Corporation
1001 West Saint Paul Ave, Milwaukee, WI 53233 USA

id 5/92- 7/92

		Sigma	Aldrich
For Emergency	Contact USA/Canada	800-325-5832	800-231-8327
	Outside USA/Canada	314-771-5765	414-273-3850



---------- IDENTIFICATION ----------

PRODUCT #: D2445-5 NAME: 3,4-DIAMINOPYRIDINE, 98%
CAS #: 54-96-6
MF: C5H7N3

SYNONYMS
3,4-DIAMINOPYRIDINE * DIAMINO-3,4 PYRIDINE * SC10 *

---------- TOXICITY HAZARDS ----------

RTECS NO: US7600000
PYRIDINE, 3,4-DIAMINO-

TOXICITY DATA

IPR-MUS LD50:20 MG/KG	JMCMAR 8,296,65
SCU-MUS LD50:35 MG/KG	AIPTAK 150,413,64
IVN-MUS LD50:13 MG/KG	APFRAD 26,345,68
ORL-BWD LD50:75 MG/KG	AECTCV 12,355,83

TARGET ORGAN DATA
BEHAVIORAL (CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD)
BEHAVIORAL (CHANGE IN MOTOR ACTIVITY)
LUNGS, THORAX OR RESPIRATION (RESPIRATORY STIMULATION)
GASTROINTESTINAL (CHANGES IN STRUCTURE OR FUNCTION OF SALIVARY GLANDS)
SKIN AND APPENDAGES (HAIR)
ONLY SELECTED REGISTRY OF TOXIC EFFECTS OF CHEMICAL SUBSTANCES (RTECS) DATA IS PRESENTED HERE. SEE ACTUAL ENTRY IN RTECS FOR COMPLETE INFORMATION.

---------- HEALTH HAZARD DATA ----------

ACUTE EFFECTS

HARMFUL IF SWALLOWED, INHALED, OR ABSORBED THROUGH SKIN.
CAUSES EYE AND SKIN IRRITATION.
MATERIAL IS IRRITATING TO MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT.
TO THE BEST OF OUR KNOWLEDGE, THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED.

FIRST AID

IN CASE OF CONTACT, IMMEDIATELY FLUSH EYES WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES.
IN CASE OF CONTACT, IMMEDIATELY WASH SKIN WITH SOAP AND COPIOUS AMOUNTS OF WATER.
IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN.
IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS.
CALL A PHYSICIAN.
WASH CONTAMINATED CLOTHING BEFORE REUSE.

---------- PHYSICAL DATA ----------

MELTING PT: 218 C TO 220 C

APPEARANCE AND ODOR

LIGHT-TAN POWDER

---------- FIRE AND EXPLOSION HAZARD DATA ----------

EXTINGUISHING MEDIA

WATER SPRAY.
CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM.

SPECIAL FIREFIGHTING PROCEDURES

WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO PREVENT CONTACT WITH SKIN AND EYES.

---------- REACTIVITY DATA ----------

INCOMPATIBILITIES

STRONG OXIDIZING AGENTS

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS

TOXIC FUMES OF:
CARBON MONOXIDE, CARBON DIOXIDE
NITROGEN OXIDES

---------- SPILL OR LEAK PROCEDURES ----------

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED

WEAR SELF-CONTAINED BREATHING APPARATUS, RUBBER BOOTS AND HEAVY RUBBER GLOVES.
SWEEP UP, PLACE IN A BAG AND HOLD FOR WASTE DISPOSAL.
AVOID RAISING DUST.
VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE.

WASTE DISPOSAL METHOD

DISSOLVE OR MIX THE MATERIAL WITH A COMBUSTIBLE SOLVENT AND BURN IN A CHEMICAL INCINERATOR EQUIPPED WITH AN AFTERBURNER AND SCRUBBER.
OBSERVE ALL FEDERAL, STATE, AND LOCAL LAWS.

--- PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE ---

CHEMICAL SAFETY GOGGLES.
RUBBER GLOVES.
NIOSH/MSHA-APPROVED RESPIRATOR.
SAFETY SHOWER AND EYE BATH.
MECHANICAL EXHAUST REQUIRED.
DO NOT BREATHE DUST.
DO NOT GET IN EYES, ON SKIN, ON CLOTHING.
WASH THOROUGHLY AFTER HANDLING.

TOXIC.
IRRITANT.
KEEP TIGHTLY CLOSED.
STORE IN A COOL DRY PLACE.
TOXIC BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED.
IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.
WEAR SUITABLE PROTECTIVE CLOTHING.

mine. Miosis occurs within 10 to 20 minutes of instillation of carbachol eye drops and lasts for 4 to 8 hours; reduction in intra-ocular pressure lasts for 8 hours.
Carbachol is also administered intra-ocularly, 0.4 to 0.5 mL of a 0.01% solution being instilled into the anterior chamber of the eye, to produce miosis in cataract surgery. The maximum degree of miosis is usually obtained within 2 to 5 minutes of intra-ocular instillation and miosis lasts for 24 to 48 hours.
Carbachol has been used as an alternative to catheterisation in the treatment of urinary retention in a dose of 2 mg given three times daily by mouth. For the acute symptoms of postoperative urinary retention doses of 250 µg have been given subcutaneously repeated twice if necessary at 30-minute intervals. Carbachol should not be given by the intravenous or intramuscular routes.

Carbachol does not readily penetrate the cornea and eye drops are usually prepared with a wetting agent to enhance penetration. A lipid-soluble derivative, N-demethylated carbachol has been studied for use in glaucoma.[1]

1. Hung PT, *et al.* Ocular hypotensive effects of N-demethylated carbachol on open angle glaucoma. *Arch Ophthalmol* 1982; **100**: 262–4.

Ocular surgery. Some consider carbachol to be the agent of choice for the management of increased intra-ocular pressure after cataract extraction.[1,2]

1. Ruiz RS, *et al.* Effects of carbachol and acetylcholine on intraocular pressure after cataract extraction. *Am J Ophthalmol* 1989; **107**: 7–10.
2. Hollands RH, *et al.* Control of intraocular pressure after cataract extraction. *Can J Ophthalmol* 1990; **25**: 128–32.

Urinary incontinence. For a discussion on the use of parasympathomimetics in the management of urinary incontinence, see under Uses and Administration of Bethanechol Chloride, p.1113.

Proprietary Names
Carbamann, Doryl, Isopto Karbakolin, Miostat, Spersacarbachol.
Multi-ingredient preparations. Bestrolina, GT 50, Mios, Risunal A, Risunal B.
Preparation details are given in Part 3.

Choline Alfoscerate (4848-h)

Choline Alfoscerate *(rINN)*.
Choline Glycerophosphate; L-α-Glycerylphosphorylcholine. Choline hydroxide, (*R*)-2,3-dihydroxypropyl hydrogen phosphate, inner salt.
$C_8H_{20}NO_6P$ = 257.2.

CAS — 28319-77-9.

Choline alfoscerate is reported to have cholinergic activity and has been tried by intravenous or intramuscular administration in the treatment of Alzheimer's disease and other dementias.

References.
1. Trabucchi M, *et al.* Changes in the interactions between CNS cholinergic and dopaminergic neurons induced by L-α-glycerylphosphorylcholine, a cholinomimetic drug. *Farmaco (Sci)* 1986; **41**: 323–34.
2. Di Perri R, *et al.* A multicentre trial to evaluate the efficacy and tolerability of α-glycerylphosphorylcholine versus cytosine diphosphocholine in patients with vascular dementia. *J Int Med Res* 1991; **19**: 330–41.

Proprietary Names
Brezal, Delecit, Gliatilin.
Preparation details are given in Part 3.

Demecarium Bromide (4512-n)

Demecarium Bromide *(BAN, rINN)*.
BC-48. *N,N'*-Decamethylenebis(*N,N,N*-trimethyl-3-methylcarbamoyloxyanilinium) dibromide.
$C_{32}H_{52}Br_2N_4O_4$ = 716.6.

CAS — 56-94-0.

Pharmacopoeias. In *U.S.*

A white or slightly yellow, slightly hygroscopic, crystalline powder. Freely **soluble** in water and alcohol; soluble in ether; sparingly soluble in acetone. A 1% solution in water has a pH of 5 to 7. **Store** in airtight containers. Protect from light.

Adverse Effects
As for Neostigmine Methylsulphate, p.1116 and Ecothiopate Iodide, below. The anticholinesterase action of demecarium, and hence its adverse effects, may be prolonged.

Treatment of Adverse Effects and Precautions
As for Ecothiopate Iodide, p.1115.
Pralidoxime has been reported to be more active in counteracting the effects of dyflos and ecothiopate than of demecarium.

Uses and Administration
Demecarium is a quaternary ammonium compound which is an inhibitor of cholinesterase with actions similar to those of ecothiopate (see below). Its miotic action begins within about 15 to 60 minutes of its application and may persist for a week or more. It causes a reduction in intra-ocular pressure which is maximal in 24 hours and may persist for 9 days or more.
Demecarium bromide has been used in the treatment of open-angle glaucoma particularly in aphakic patients, and those in whom other agents have proved inadequate. The dosage varies, 1 to 2 drops of a 0.125% or 0.25% solution being instilled from twice weekly to twice daily, preferably at bedtime.
Demecarium bromide has also been used in the diagnosis and management of accommodative convergent strabismus (esotropia).

Proprietary Names
Humorsol, Tosmilen.
Preparation details are given in Part 3.

3,4-Diaminopyridine (19064-m)

3,4-Diaminopyridine has similar actions and uses to 4-aminopyridine (see p.1112) but is reported to be more potent in enhancing the release of acetylcholine from nerve terminals.

Administration of 3,4-diaminopyridine by mouth in daily doses of up to 100 mg in a double-blind, placebo-controlled, crossover study was found to be effective in the treatment of both the motor and autonomic deficits of 12 patients with Eaton-Lambert syndrome. One patient receiving 100 mg daily had a single seizure after 10 months of therapy but adverse effects in other patients were minimal and dose-related. In 4 patients addition of pyridostigmine to treatment produced additional benefits.— McEvoy KM, *et al.* 3,4-Diaminopyridine in the treatment of Lambert-Eaton myasthenic syndrome. *N Engl J Med* 1989; **321**: 1567–71.

Distigmine Bromide (4513-h)

Distigmine Bromide *(BAN, rINN)*.
BC-51; Bispyridostigmine Bromide; Hexamarium Bromide. 3,3'-[N,N'-Hexamethyelenebis(methylcarbamoyloxy)]bis(1-methylpyridinium bromide).
$C_{22}H_{32}Br_2N_4O_4$ = 576.3.

CAS — 15876-67-2.

Pharmacopoeias. In *Jpn.*

Adverse Effects, Treatment, and Precautions
As for Neostigmine, p.1116. The anticholinesterase action of distigmine, and hence its adverse effects, may be prolonged, and if treatment with atropine is required it should be maintained for at least 24 hours.

Absorption and Fate
Distigmine is poorly absorbed from the gastro-intestinal tract.

Uses and Administration
Distigmine is a quaternary ammonium compound which is an inhibitor of cholinesterase activity with actions similar to those of neostigmine (see p.1117) but more prolonged. Maximum inhibition of plasma cholinesterase occurs 9 hours after a single intramuscular dose, and persists for about 24 hours.
It is used in the prevention and treatment of postoperative intestinal atony and urinary retention; 500 µg of distigmine bromide may be injected intramuscularly about 12 hours after surgery and may be repeated every 24 hours until normal function is restored. It may also be given by mouth in a dose of 5 mg daily thirty minutes before breakfast. A similar dose by mouth, given daily or on alternate days, has been employed in the management of neurogenic bladder.
Distigmine bromide in conjunction with short-acting parasympathomimetics has been given for the treatment of myasthenia gravis, but should only be given by mouth. Doses of up to 20 mg daily for adults and up to 10 mg daily for children have been used, adjusted according to individual response.

Proprietary Names
Ubretid.
Preparation details are given in Part 3.

Dyflos (4514-m)

Dyflos *(BAN)*.
DFP; Difluorophate; Di-isopropyl Fluorophosphate; Di-isopropylfluorophosphonate; Fluostigmine; Isoflurophate; isopropyl phosphorofluoridate.
$C_6H_{14}FO_3P$ = 184.1.

CAS — 55-91-4.

Pharmacopoeias. In *U.S.*

A clear, colourless, or faintly yellow liquid. Specific gravity about 1.05. Sparingly **soluble** in water; soluble in alcohol and vegetable oils. It is decomposed by water with the evolution of hydrogen fluoride. **Store** at 15° in sealed containers.

CAUTION. *The vapour of dyflos is very toxic. Concentrated material should be immersed in a 2% aqueous solution of sodium hydroxide for several hours. Dyflos can be removed from the skin by washing with soap and water.*

Adverse Effects
As for Neostigmine Methylsulphate, p.1116 and Ecothiopate Iodide, below.
The anticholinesterase action of dyflos, and hence its adverse effects, may be prolonged. Its vapour is highly irritating to the eye and mucous membranes. Systemic toxicity also occurs after inhalation of the vapour. Prolonged use of dyflos in the eye may cause partly reversible depigmentation of the lid margins in dark-skinned patients

Treatment of Adverse Effects and Precautions
As for Ecothiopate Iodide, p.1115.

Absorption and Fate
Dyflos is readily absorbed from the gastro-intestinal tract, from skin and mucous membranes, and from the lungs. Dyflos interacts with cholinesterases producing a stable phosphonylated and phosphorylated derivatives which are then hydrolysed by phosphorylphosphatases. The products of hydrolysis are excreted mainly in the urine.

Uses and Administration
Dyflos is an irreversible inhibitor of cholinesterase with actions similar to those of ecothiopate (see p.1115). Dyflos has a powerful miotic action which begins within 10 minutes and may persist for up to 4 weeks. It causes a reduction in intra-ocular pressure which is maximal in 24 hours and may persist for a week.
Dyflos is used mainly in the treatment of chronic glaucoma particularly in aphakic patients, and those in whom other agents have proved inadequate. It is also employed in the diagnosis and management of accommodative convergent strabismus (esotropia).
Dyflos is administered locally usually as a 0.1% ophthalmic ointment preferably at night before retiring.

Proprietary Names
Diflupyl, Floropryl.
Preparation details are given in Part 3.

Ecothiopate Iodide (4515-b)

Ecothiopate iodide is an irreversible cholinesterase with a prolonged duration of action. It is used as a miotic in the treatment of glaucoma when other agents have proved inadequate.

Ecothiopate Iodide *(BAN, rINN)*.
Echothiophate Iodide; Ecostigmine Iodide; (2-Diethoxyphosphinylthioethyl)-trimethylammonium iodide.
$C_9H_{23}INO_3PS$ = 383.2.

CAS — 6736-03-4 (ecothiopate); 513-10-0 (ecothiopate iodide).

Pharmacopoeias. In *Br., Gr., Jpn.* and *U.S.*

A white crystalline hygroscopic powder with an odour. **Soluble** 1 in 1 of water, 1 in 25 of alcohol, in 3 of methyl alcohol; practically insoluble in organic solvents. A solution in water has a pH of 5 to 6. The *B.P.* requires **storage** between 2° and 8°; the *U.S.* requires storage preferably at a temperature of 4°. Store in airtight containers. Protect from light.

Adverse Effects
As for Neostigmine Methylsulphate, p.1116.
Ecothiopate is an irreversible cholinesterase inhibitor; its action, and hence its adverse effects, may be prolonged.
Plasma and erythrocyte cholinesterases may be diminished by treatment with eye drops of ecothiopate, or other long-acting anticholinesterases. Systemic toxicity occurs more frequently than with shorter-acting miotics. Acute iritis, retinal detachment, or precipitation of acute glaucoma may occasionally follow treatment with ecothiopate. Iris cysts (especially in children) or lens opacities may develop following prolonged treatment

...bakolin; *Switz.:* Doryl; Miostat; Spersacarbachol; *USA:* ...Miostat.

...redient preparations. *Ger.:* GT 50†; *Ital.:* Mios; ...trolina†; Risunal A; Risunal B.

...line Alfoscerate (4848-h)

...Alfoscerate *(rINN).*

...Glycerophosphate; L-α-Glycerylphosphorylcholine, ...hydroxide, (*R*)-2,3-dihydroxypropyl hydrogen phos...ner salt.

...NO_6P = 257.2.

...28319-77-9.

...f alfoscerate is reported to be a precursor of ...choline and has been tried by oral, intrave... or intramuscular administration in the treat... of Alzheimer's disease and other dementias, ...gh as mentioned in the discussion on the treat... of dementia (see p.1413) this type of treatment ...considered to produce useful improvement.

...ences.

...cchi M, *et al.* Changes in the interactions between CNS ...ergic and dopaminergic neurons induced by L-α-glycer...phorylcholine, a cholinomimetic drug. *Farmaco (Sci)* ...**41:** 323–34.

...Parri R, *et al.* A multicentre trial to evaluate the efficacy and ...bility of α-glycerylphosphorylcholine versus cytosine di...phocholine in patients with vascular dementia. *J Int Med* ...**1991; 19:** 330–41.

...parations

...of preparations are listed below; details are given in Part 3.

...rietary Preparations

...Dezral; Delecit; Gliatilin.

...emecarium Bromide (4512-n)

...carium Bromide *(BAN, rINN).*

...*N,N'*-Decamethylenebis(*N,N,N*-trimethyl-3-methyl...moyloxyanilinium) dibromide.

...$_{32}Br_2N_4O_4$ = 716.6.

...56-94-0.

...copoeias. In *US.*

...e or slightly yellow, slightly hygroscopic, crystalline ...r. Freely **soluble** in water and in alcohol; soluble in ...; sparingly soluble in acetone. A 1% solution in water ...pH of 5 to 7. **Store** in airtight containers. Protect from ...ht.

...verse Effects

...for Neostigmine, p.1422 and Ecothiopate Iodide, ...420. For adverse effects of miotics, see also Pilo...pine, p.1426.

...atment of Adverse Effects

...for Ecothiopate Iodide, p.1420.

...idoxime has been reported to be more active in ...teracting the effects of dyflos and ecothiopate ...of demecarium.

...recautions

...for Neostigmine, p.1423 and Ecothiopate Iodide, ...420. For precautions of miotics, see also Pilo...pine, p.1426.

...es and Administration

...emecarium is a quaternary ammonium compound ...ch is a reversible inhibitor of cholinesterase with ...long duration of action similar to that of ecothio... iodide (see p.1420). Its miotic action begins ...within about 15 to 60 minutes of its application and ...may persist for a week or more. It causes a reduction ...in intra-ocular pressure which is maximal in 24 ...urs and may persist for 9 days or more.

...emecarium bromide has been used in the treatment ...of open-angle glaucoma particularly in aphakic pa...ents, and those in whom other agents have proved ...adequate (see p.1414). The dosage varies, 1 to 2 ...drops of a 0.125% or 0.25% solution being instilled ...from twice weekly to twice daily, preferably at bed...me. Demecarium bromide has also been used in the ...diagnosis and management of accommodative convergent strabismus (accommodative esotropia) as mentioned in the discussion on the treatment of strabismus on p.1416.

Preparations

Names of preparations are listed below; details are given in Part 3.

Official Preparations

USP 23: Demecarium Bromide Ophthalmic Solution.

Proprietary Preparations

UK: Tosmilen†; *USA:* Humorsol.

3,4-Diaminopyridine (19064-m)

3,4-Diaminopyridine has similar actions and uses to fampridine (see p.1421) but is reported to be more potent in enhancing the release of acetylcholine from nerve terminals. It is used in the Eaton-Lambert myasthenic syndrome and other myasthenic conditions. It has been tried in multiple sclerosis and in botulism.

No improvement was observed with 3,4-diaminopyridine in a controlled study of patients with chronic demyelinating neuropathy.[1]

1. Russell JW, *et al.* Treatment of stable chronic demyelinating polyneuropathy with 3,4-diaminopyridine. *Mayo Clin Proc* 1995; **70:** 532–9.

Eaton-Lambert myasthenic syndrome. Administration of 3,4-diaminopyridine by mouth in daily doses of up to 100 mg has been found to be effective in the treatment of both the motor and autonomic deficits of patients with Eaton-Lambert syndrome.[1] A usual starting dose of 10 mg given three or four times daily increasing if necessary to a maximum of 20 mg given five times daily has been used.[2] Adverse effects appear to be mainly mild and dose related.[1] Most patients experience some form of paraesthesia up to 60 minutes after administration.[1,2] 3,4-Diaminopyridine can produce mild excitatory effects and some patients may experience difficulty in sleeping. There have been isolated reports of seizures and 3,4-diaminopyridine is therefore contra-indicated in patients with epilepsy. Other treatments of Eaton-Lambert myasthenic syndrome are discussed on p.1414.

1. McEvoy KM, *et al.* 3,4-Diaminopyridine in the treatment of Lambert-Eaton myasthenic syndrome. *N Engl J Med* 1989; **321:** 1567–71.
2. Newsom-Davis J. Myasthenia gravis and the Lambert-Eaton myasthenic syndrome. *Prescribers' J* 1993; **33:** 205–212.

Distigmine Bromide (4513-h)

Distigmine Bromide *(BAN, rINN).*

BC-51; Bispyridostigmine Bromide; Hexamarium Bromide.

3,3'-[N,N'-Hexamethylenebis(methylcarbamoyloxy)]bis(1-methylpyridinium bromide).

$C_{22}H_{32}Br_2N_4O_4$ = 576.3.

CAS — 15876-67-2.

Pharmacopoeias. In *Jpn.*

Adverse Effects, Treatment, and Precautions

As for Neostigmine, p.1422. The anticholinesterase action of distigmine, and hence its adverse effects, may be prolonged, and if treatment with atropine is required it should be maintained for at least 24 hours.

Pharmacokinetics

Distigmine is poorly absorbed from the gastro-intestinal tract.

Uses and Administration

Distigmine is a quaternary ammonium compound which is a reversible inhibitor of cholinesterase activity with actions similar to those of neostigmine (see p.1423) but more prolonged. Maximum inhibition of plasma cholinesterase occurs 9 hours after a single intramuscular dose, and persists for about 24 hours.

It is one of several agents that may be used in the prevention and treatment of postoperative intestinal atony (see p.1193). It is also used in urinary retention, although catheterisation is generally preferred (see p.489). A dose of 500 μg of distigmine bromide may be injected intramuscularly about 12 hours after surgery and may be repeated every 24 hours until normal function is restored. It may also be given by mouth in a dose of 5 mg daily thirty minutes before breakfast. A similar dose by mouth, given daily or on alternate days, has been employed in the management of neurogenic bladder.

Distigmine bromide in conjunction with short-acting parasympathomimetics is also used for the treatment of myasthenia gravis, but should only be given by mouth. Also, as discussed under the section on the treatment of myasthenia gravis, patients being treated with parasympathomimetics tend to prefer pyridostigmine (see p.1415). Doses of up to 20 mg daily for adults and up to 10 mg daily for children are given, adjusted according to individual response.

Preparations

Names of preparations are listed below; details are given in Part 3.

Proprietary Preparations

Aust.: Ubretid; *Austral.:* Ubretid; *Eire:* Ubretid; *Ger.:* Ubretid; *Neth.:* Ubretid; *S.Afr.:* Ubretid; *Switz.:* Ubretid; *UK:* Ubretid.

Dyflos (4514-m)

Dyflos *(BAN).*

DFP; Difluorophate; Di-isopropyl Fluorophosphate; Di-isopropylfluorophosphonate; Fluostigmine; Isoflurophate. Di-isopropyl phosphorofluoridate.

$C_6H_{14}FO_3P$ = 184.1.

CAS — 55-91-4.

Pharmacopoeias. In *US.*

A clear, colourless, or faintly yellow liquid. Specific gravity about 1.05. Sparingly **soluble** in water; soluble in alcohol and in vegetable oils. It is decomposed by moisture with the evolution of hydrogen fluoride. **Store** at 8° to 15° in sealed containers.

CAUTION. *The vapour of dyflos is very toxic. The eyes, nose, and mouth should be protected when handling dyflos, and contact with the skin should be avoided. Dyflos can be removed from the skin by washing with soap and water. Contaminated material should be immersed in a 2% aqueous solution of sodium hydroxide for several hours.*

Adverse Effects

As for Neostigmine Methylsulphate, p.1422 and Ecothiopate Iodide, p.1420. For adverse effects of miotics, see also Pilocarpine, p.1426.

The anticholinesterase action of dyflos, and hence its adverse effects, may be prolonged. Its vapour is extremely irritating to the eye and mucous membranes.

Systemic toxicity also occurs after inhalation of the vapour. Prolonged use of dyflos in the eye may cause slowly reversible depigmentation of the lid margins in dark-skinned patients.

Treatment of Adverse Effects

As for Ecothiopate Iodide, p.1420.

Precautions

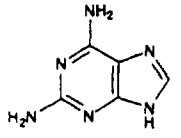
As for Neostigmine, p.1423 and Ecothiopate Iodide, p.1420. For precautions of miotics, see also Pilocarpine, p.1426.

Pharmacokinetics

Dyflos is readily absorbed from the gastro-intestinal tract, from skin and mucous membranes, and from the lungs. Dyflos interacts with cholinesterases producing stable phosphonylated and phosphorylated derivatives which are then hydrolysed by phosphorylphosphatases. These products of hydrolysis are excreted mainly in the urine.

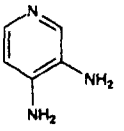
Uses and Administration

Dyflos is an irreversible inhibitor of cholinesterase with actions similar to those of ecothiopate iodide (see p.1420). Dyflos has a powerful miotic action which begins within 5 to 10 minutes and may persist for up to 4 weeks; it causes a reduction in intra-ocular pressure which is maximal in 24 hours and may persist for a week.



Crystals from ethanol + water, mp 302°. uv max (pH 1.9): 241, 282 nm (log ε 3.98, 4.00).

3029. 3,4-Diaminopyridine. *3,4-Pyridinediamine;* 3,4-DAP. $C_5H_7N_3$; mol wt 109.13. C 55.03%, H 6.47%, N 38.50%. Potassium channel blocker; antagonizes non-depolarizing neuromuscular blockade. Prepn: O. Bremer, *Ann.* **518**, 274 (1935); J. W. Clark-Lewis, R. P. Singh, *J. Chem. Soc.* **1962**, 2379; J. B. Campbell *et al., J. Heterocycl. Chem.* **23**, 669 (1986). HPLC determn in serum: J. Leslie, C. T. Bever, *J. Chromatog.* **496**, 214 (1989). Acute toxicity: P. Lechat *et al., Ann. Pharm. Franc.* **26**, 345 (1968). Effect on neuromuscular transmission: J. Molgo *et al., Eur. J. Pharmacol.* **61**, 25 (1980); R. H. Thomsen, D. F. Wilson, *J. Pharmacol. Exp. Ther.* **227**, 260 (1983). Evaluation in human botulism: A. P. Ball *et al., Quart. J. Med.* **48**, 473 (1979). Clinical evaluations in myasthenic syndromes: K. M. McEvoy *et al., N. Engl. J. Med.* **321**, 1567 (1989); J. Palace *et al., J. Neurol. Neurosurg. Psychiatr.* **54**, 1069 (1991); in multiple sclerosis: C. T. Bever, Jr. *et al., Ann. Neurol.* **27**, 421 (1990); *idem, ibid.* **36**, S118 (1994).



Needles from water, mp 220° (Clark-Lewis, Singh); also reported as white to beige crystals from water, mp 218-219° (Campbell). Readily sol in water, alcohol; slightly sol in ether. LD_{50} i.v. in mice: 13 mg/kg (Lechat).

USE: Intermediate in synthesis of heterocyclic compds.

3030. Diamond. A crystalline form of carbon. Mined as a mineral, principally in South Africa. (Non-commercial) synthesis from other carbon compds (e.g., lignin) by means of elevated temperatures (about 2700°) and pressures (about 800,000 lbs/sq inch): Desch, *Nature* **152**, 148 (1943); Neuhaus, *Angew. Chem.* **66**, 525 (1954); Hall, *Chem. Eng. News* **33**, 718 (1955); Bridgman, *Sci. Amer.* **1955**, 46; Hall, *J. Chem. Ed.* **38**, 484 (1961); Bundy, *Ann. N.Y. Acad. Sci.* vol. **105**, art 17, pp 951-982 (1964). Books: S. Tolansky, *History and Use of Diamond* (London, 1962) 166 pp; R. Berman, *Physical Properties of Diamond* (Oxford, 1965) 442 pp.

Face-centered cubic crystal lattice. Burns when heated with a hot enough flame (over 800°, oxygen torch). d_4^{25} 3.513. n_D^{20} 2.4173. Hardness = 10 (Mohs' scale). Sp heat at 100°K: 0.606 cal/g-atom/°K. Entropy at 298.16°K: 0.5684 cal/g-atom/°K. Band gap energy: 6.7 ev. Dielectric constant 5.7. Electron mobility: ~1800 cm^2/v-sec. Hole mobility: 1200 cm^2/v-sec. Can be pulverized in a steel mortar. Attacked by laboratory-type cleaning soln (potassium dichromate + concd H_2SO_4). In the jewelry trade the unit of weight for diamonds is one carat = 200 mg. *Ref: Wall Street J.* **164**, no. 36, p 10 (Aug 19, 1964).

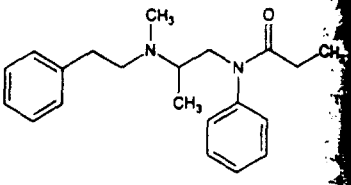
USE: Jewelry. Polishing, grinding, cutting glass, bearings for delicate instruments; manuf dies for tungsten wire and similar hard wires; making styli for recorder heads, long-lasting phonograph needles. In semiconductor research.

3031. Diamond Ink. Etching ink. A mixture of HF, $BaSO_4$ and fluorides.

Milky-white liq with a heavy sediment. Shake well before using and warm gently in a lead dish. *Keep in plastic, hard-rubber or intern. paraffin-coated bottles.*

USE: For etching glass.

3032. Diampromide. *N-[2-[Methyl(2-phenylethyl)amino]propyl]-N-phenylpropanamide; N-[2-(methylphenethylamino)propyl]propionanilide.* $C_{21}H_{28}N_2O$; mol wt 324.47. C 77.74%, H 8.70%, N 8.63%, O 4.93%. Synthesis: Wright *et al., J. Am. Chem. Soc.* **81**, 1518 (1959); U.S. pat. (1960 to American Cyanamid).



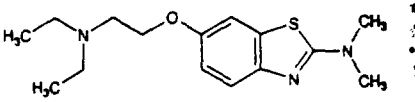
Liquid, $bp_{0.5}$ 174-178°. n_D^{25} 1.546.

Sulfate, $C_{21}H_{30}N_2O_5S$, crystals from ethanol + ether, mp 110-111°.

Note: This is a controlled substance (opiate) listed in U.S. Code of Federal Regulations, Title 21 Part 1308.11 (1995).

THERAP CAT: Analgesic (narcotic).

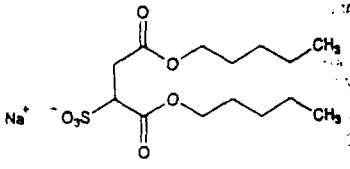
3033. Diamthazole Dihydrochloride. *6-[2-(Diethylamino)ethoxy]-N,N-dimethyl-2-benzothiazolamine dihydrochloride; 6-[2-(diethylamino)ethoxy]-2-dimethylaminobenzothiazole dihydrochloride;* 2-dimethylamino-6-(β-diethylaminoethoxy)benzothiazole dihydrochloride; dimazole dihydrochloride; Ro-2-2453; Asterol Dihydrochloride; Ateb. $C_{15}H_{25}Cl_2N_3OS$; mol wt 366.35. C 49.18%, H 6.88%, Cl 19.35%, N 11.47%, O 4.37%, S 8.75%. Prepn: Steinmeyer, U.S. pat. 2,578,757 (1951 to Hoffmann-La Roche).



Crystals, dec 269°, mp 240-243°. Freely sol in methanol, ethanol. A 5% aq soln has a pH of ~2.

THERAP CAT: Antifungal.

3034. Diamyl Sodium Sulfosuccinate. *Sulfosuccinic acid 1,4-dipentyl ester sodium salt; sulfosuccinic acid 1,4-dipentyl ester sodium salt;* Aerosol AY; Alphasol AY; $C_{14}H_{25}NaO_7S$; mol wt 360.40. C 46.66%, H 6.99%, Na 6.38%, O 31.08%, S 8.90%. The amyl or 1-methylbutyl diester, monosodium salt of sulfosuccinic acid or a mixture. Wetting agent prepd by the action of the appropriate alcohols on maleic anhydride followed by addtion of sodium sulfite: Jaeger, U.S. pats. 2,028,091 and 2,176,423 (both 1939 to Am. Cyanamid).



Available as a mixture of white, hard pellets and flakes. Soly in water at 25° = 392 g/liter; at 70° = 502 g/liter. Maximum concn of electrolyte soln in which 1% of the wetting agent is sol: 3% NaCl; 2-4% NH_4Cl (turbid); 2% $(NH_4)_2HPO_4$ (turbid); 4% $NaNO_3$ (slightly turbid); 4% Na_2SO_4 (very slightly turbid). Also sol in pine oil, acetic acid, acetone, hot kerosene, carbon tetrachloride, benzene, hot olive oil; insol in liq petrolatum. Surface tension in water: 0.001% = 69.4 dyn/cm; 0.02% = 68.3 dyn/cm; 0.1% = 50.2 dyn/cm; 0.25% = 41.6 dyn/cm; 1% = 32.5 dyn/cm. Interfacial tension 1% in water *vs.* liquid petrolatum: 5 seconds = 7.55 dyn/cm; 30 seconds = 7.23 dyn/cm; 15 minutes = 7.03 dyn/cm. Interfacial tension 0.1% in water *vs.* liquid petrolatum: 5 seconds = 29.6 dyn/cm; 30 seconds = 28.6 dyn/cm; 15 minutes = 28.2 dyn/cm. Stable in acid and neutral solns, hydrolyzes in alkaline solns.

USE: As emulsifier in emulsion polymerization; as wetting agent.

3035. 1,2-Dianilinoethane. *N,N'-Diphenyl-1,2-ethanediamine; N,N'-diphenylethylenediamine; N,N'-diphe*

National Library of Medicine: IGM Full Record Screen

TITLE: Treatment with oral 3,4 diaminopyridine improves leg strength in multiple sclerosis patients: results of a randomized, double-blind, placebo-controlled, crossover trial.

AUTHOR: Bever CT Jr; Anderson PA; Leslie J; Panitch HS; Dhib-Jalbut S; Khan OA; Milo R; Hebel JR; Conway KL; Katz E; Johnson KP

AUTHOR AFFILIATION: Department of Neurology, School of Medicine, University of Maryland, Baltimore, USA.

SOURCE: Neurology 1996 Dec;47(6):1457-62

NLM CIT. ID: 97120056

ABSTRACT: To examine the efficacy and toxicity of oral 3,4 diaminopyridine (DAP) in dosages up to 100 mg/day, 36 patients with multiple sclerosis (MS) enrolled in a randomized, double-blind, placebo-controlled, crossover trial. The primary outcome measure was improvement of a prospectively defined neurologic deficit, which was leg weakness in 34 patients. Secondary outcome measures included the patient's subjective response, scored manual motor testing (MMT) of leg strength, scored leg strength from videotaped motor testing (VMT), quadriceps and hamstrings strength (QMT) measured by isometric dynamometry, neuropsychological testing (NPT), ambulation index (AI), and Expanded Disability Status Scale (EDSS) score. Paresthesias and abdominal pain were common and were dose limiting in eight patients. Three patients had episodes of confusion, and one patient had a seizure while on DAP. Eight patients withdrew from the study, leaving 28 evaluable patients for the efficacy analysis. The prospectively defined neurologic deficit improved in 24 patients-22 on DAP and 2 on placebo ($p = 0.0005$). All improvements were in leg weakness. Subjective response and measures of leg strength and function (MMT, VMT, QMT, and AI) improved on DAP compared with placebo. Neither NPT nor EDSS scores improved. DAP treatment can induce improvements in leg strength in MS patients, but toxicity is limiting in many patients.

MAIN MESH SUBJECTS: Leg/*PHYSIOPATHOLOGY
Multiple Sclerosis/*DRUG THERAPY/PHYSIOPATHOLOGY
4-Aminopyridine/*ANALOGS & DERIVATIVES/ADMINISTRATION & DOSAGE

http://130.14.32.44/cgi-bin/IGM-client?5360+detail+1

ADDITIONAL MESH SUBJECTS:	**Administration, Oral** **Adult** **Aged** **Double-Blind Method** **Female** **Human** **Male** **Middle Age** **Support, Non-U.S. Gov't**
PUBLICATION TYPES:	**CLINICAL TRIAL** **JOURNAL ARTICLE** **RANDOMIZED CONTROLLED TRIAL**
LANGUAGE:	**Eng**
REGISTRY NUMBERS:	**504-24-5 (4-Aminopyridine)** **54-96-6 (3,4-diaminopyridine)**

National Library of Medicine: IGM Full Record Screen

TITLE:	Low-dose guanidine and pyridostigmine: relatively safe and effective long-term symptomatic therapy in Lambert-Eaton myasthenic syndrome.
AUTHOR:	Oh SJ; Kim DS; Head TC; Claussen GC
AUTHOR AFFILIATION:	Department of Neurology, University of Alabama at Birmingham 35294, USA.
SOURCE:	Muscle Nerve 1997 Sep;20(9):1146-52
NLM CIT. ID:	97416721
ABSTRACT:	Guanidine hydrochloride is known to be highly effective in the symptomatic treatment of the Lambert-Eaton myasthenic syndrome (LEMS). However, because of its potentially dangerous side reactions of hematologic abnormalities and renal insufficiency, 3,4-diaminopyridine, which is not readily available in the United States, is recommended as the preferred drug for LEMS. We used low-dose guanidine and pyridostigmine combination therapy in 9 patients with LEMS and analyzed its long-term safety and effectiveness. In all patients, a liberal amount of pyridostigmine was used, while daily guanidine dose was kept below 1000 mg a day, and guanidine was given between pyridostigmine dosings. This combination therapy was used for 3-102 months (mean: 34.1 months) and improved clinical status in all patients. Although guanidine had to be discontinued due to severe gastrointestinal symptoms in 3 cases, no serious side reactions such as bone marrow suppressions or signs of renal insufficiency developed in any case. Thus, we conclude that low-dose guanidine therapy is relatively safe and effective for long-term symptomatic treatment of LEMS when it is combined with pyridostigmine.
MAIN MESH SUBJECTS:	Cholinesterase Inhibitors/*ADMINISTRATION & DOSAGE/ADVERSE EFFECTS/THERAPEUTIC USE Guanidines/*ADMINISTRATION & DOSAGE/ADVERSE EFFECTS/THERAPEUTIC USE Lambert-Eaton Myasthenic Syndrome/COMPLICATIONS/*DRUG THERAPY/ PHYSIOPATHOLOGY Pyridostigmine Bromide/*ADMINISTRATION & DOSAGE/ADVERSE EFFECTS/ THERAPEUTIC USE

ADDITIONAL MESH SUBJECTS:	**Adult** **Aged** **Dose-Response Relationship, Drug** **Electrophysiology** **Female** **Human** **Male** **Middle Age** **Neoplasms/COMPLICATIONS/THERAPY** **Time Factors** **Treatment Outcome**
PUBLICATION TYPES:	**CLINICAL TRIAL** **JOURNAL ARTICLE**
LANGUAGE:	**Eng**
REGISTRY NUMBERS:	**0 (Cholinesterase Inhibitors)** **0 (Guanidines)** **101-26-8 (Pyridostigmine Bromide)** **113-00-8 (Guanidine)**

☑

TITLE: 3,4-diaminopyridine in childhood myasthenia: double-blind, placebo-controlled trial.

AUTHOR: Anlar B; Varli K; Ozdirim E; Ertan M

AUTHOR AFFILIATION: Department of Pediatric Neurology, Hacettepe University, Ankara, Turkey.

SOURCE: J Child Neurol 1996 Nov;11(6):458-61

NLM CIT. ID: 97118599

ABSTRACT: Eleven patients with congenital and five with juvenile myasthenia gravis, aged 5 to 24 years, were given 3,4-diaminopyridine in a double-blind, placebo-controlled, crossover study. Clinical improvement was observed in 5 of 11 congenital myasthenia patients, and placebo effect, in 3 of 11. Juvenile myasthenia patients did not respond. Single-fiber electromyographic studies did not reveal any changes correlating with the clinical status of the patient. This study demonstrates the importance of double-blind and placebo-controlled studies to determine the effect of 3,4-diaminopyridine in congenital myasthenia. This drug may have different effects on various presynaptic and postsynaptic defects of neuromuscular transmission resulting in congenital myasthenia syndromes.

MAIN MESH SUBJECTS: Myasthenia Gravis/CONGENITAL/DIAGNOSIS/*DRUG THERAPY
4-Aminopyridine/*ANALOGS & DERIVATIVES/THERAPEUTIC USE

ADDITIONAL MESH SUBJECTS:
Adolescence
Adult
Child
Child, Preschool
Cross-Over Studies
Double-Blind Method
Electromyography/DRUG EFFECTS
Female
Human
Male
Neurologic Examination/DRUG EFFECTS

PUBLICATION TYPES:
CLINICAL TRIAL
JOURNAL ARTICLE
RANDOMIZED CONTROLLED TRIAL

LANGUAGE: Eng

REGISTRY NUMBERS: 504-24-5 (4-Aminopyridine)
54-96-6 (3,4-diaminopyridine)

National Library of Medicine: IGM Results Screen

Citations 25 to 32 of 57 from MEDLINE 1995-98

☐ **TITLE:** Ruthenium red, a novel enhancer of K+ currents at mouse motor nerve terminals.

AUTHOR: Lin MJ; Lin-Shiau SY

AUTHOR AFFILIATION: Institute of Pharmacology, College of Medicine, National Taiwan University, Taipei, R.O.C.

SOURCE: Neuropharmacology 1996 May;35(5):615-23

NLM CIT. ID: 97042771 (abstract present)

☑ **TITLE:** Treatment with oral 3,4 diaminopyridine improves leg strength in multiple sclerosis patients: results of a randomized, double-blind, placebo-controlled, crossover trial.

AUTHOR: Bever CT Jr; Anderson PA; Leslie J; Panitch HS; Dhib-Jalbut S; Khan OA; Milo R; Hebel JR; Conway KL; Katz E; Johnson KP

AUTHOR AFFILIATION: Department of Neurology, School of Medicine, University of Maryland, Baltimore, USA.

SOURCE: Neurology 1996 Dec;47(6):1457-62

NLM CIT. ID: 97120056 (abstract present)

☐ **TITLE:** 3,4-Diaminopyridine, an orphan drug, in the symptomatic treatment of Lambert-Eaton myasthenic syndrome.

AUTHOR: Molgo J; Guglielmi JM

AUTHOR AFFILIATION: Laboratoire de Neurobiologie Cellulaire et Moleculaire, C.N.R.S., Gif-sur-Yvette, France.

SOURCE: Pflugers Arch 1996;431(6 Suppl 2):R295-6

NLM CIT. ID: 96364143 (abstract present)

☐ **TITLE:** Acute ventilatory failure in Lambert-Eaton myasthenic syndrome and its response to 3,4-diaminopyridine.

AUTHOR: Smith AG; Wald J

AUTHOR AFFILIATION: Department of Neurology, University of Michigan Medical Center, Ann Arbor 48109, USA.
SOURCE: Neurology 1996 Apr;46(4):1143-5
NLM CIT. ID: 96373816 (abstract present)

☐ TITLE: A double-blind placebo-controlled study of 3,4-diaminopyridine in amytrophic lateral sclerosis patients on a rehabilitation unit.
AUTHOR: Aisen ML; Sevilla D; Edelstein L; Blass J
AUTHOR AFFILIATION: Burke Rehabilitation Center, White Plains, NY 10605, USA.
SOURCE: J Neurol Sci 1996 Jun;138(1-2):93-6
NLM CIT. ID: 96383381 (abstract present)

☐ TITLE: Contribution of a non-inactivating potassium current to the resting membrane potential of fusion-competent human myoblasts.
AUTHOR: Bernheim L; Liu JH; Hamann M; Haenggeli CA; Fischer-Lougheed J; Bader CR
AUTHOR AFFILIATION: Departement de Physiologie, Hopital Cantonal Universitaire, Geneva, Switzerland.
SOURCE: J Physiol (Lond) 1996 May 15;493 (Pt 1):129-41
NLM CIT. ID: 96330881 (abstract present)

☐ TITLE: 3,4-Diaminopyridine masks the inhibition of noradrenaline release from chick sympathetic neurons via presynaptic alpha 2-adrenoceptors: insights into the role of N- and L-type calcium channels.
AUTHOR: Dolezal V; Huang HY; Schobert A; Hertting G
AUTHOR AFFILIATION: Institute of Physiology, Academy of Sciences of Czech Republic, Prague 4, Czech Republic.
SOURCE: Brain Res 1996 May 20;721(1-2):101-10
NLM CIT. ID: 96385230 (abstract present)

☐ TITLE: Toosendanin facilitates [3H]noradrenaline release from rat hippocampal slices.
AUTHOR: Hua-Yu H; Cheng-Wen Z; Yu-Liang S
AUTHOR AFFILIATION: Shanghai Institute of Physiology, Chinese Academy of Sciences, China.
SOURCE: Nat Toxins 1996;4(2):92-5
NLM CIT. ID: 96289783 (abstract present)

Treatment with oral 3,4 diaminopyridine improves leg strength in multiple sclerosis patients:

Results of a randomized, double-blind, placebo-controlled, crossover trial

C.T. Bever, Jr., MD; P.A. Anderson, PhD; J. Leslie, PhD; H.S. Panitch, MD; S. Dhib-Jalbut, MD; O.A. Khan, MD; R. Milo, MD; J.R. Hebel, PhD; K.L. Conway, RN; E. Katz, RN; and K.P. Johnson, MD

Article abstract—To examine the efficacy and toxicity of oral 3,4 diaminopyridine (DAP) in dosages up to 100 mg/day, 36 patients with multiple sclerosis (MS) enrolled in a randomized, double-blind, placebo-controlled, crossover trial. The primary outcome measure was improvement of a prospectively defined neurologic deficit, which was leg weakness in 34 patients. Secondary outcome measures included the patient's subjective response, scored manual motor testing (MMT) of leg strength, scored leg strength from videotaped motor testing (VMT), quadriceps and hamstrings strength (QMT) measured by isometric dynamometry, neuropsychological testing (NPT), ambulation index (AI), and Expanded Disability Status Scale (EDSS) score. Paresthesias and abdominal pain were common and were dose limiting in eight patients. Three patients had episodes of confusion, and one patient had a seizure while on DAP. Eight patients withdrew from the study, leaving 28 evaluable patients for the efficacy analysis. The prospectively defined neurologic deficit improved in 24 patients—22 on DAP and 2 on placebo ($p = 0.0005$). All improvements were in leg weakness. Subjective response and measures of leg strength and function (MMT, VMT, QMT, and AI) improved on DAP compared with placebo. Neither NPT nor EDSS scores improved. DAP treatment can induce improvements in leg strength in MS patients, but toxicity is limiting in many patients.

NEUROLOGY 1996;47:1457–1462

Multiple sclerosis (MS) is a primary inflammatory demyelinating disease of the CNS that frequently causes chronic neurologic symptoms[1] that vary widely from patient to patient depending on the location and extent of demyelination.[2] Although symptomatic treatments are available for some MS symptoms,[3] there are no pharmacologic treatments for leg weakness, one of the most common and disabling MS symptoms. The observations that cooling[4] and changes in serum ionized calcium[5] could cause improvement of neurologic symptoms in MS patients suggested that the dysfunction was, in part, physiologic rather than being due to axonal or neuronal loss. Pathologic studies showing relative preservation of axons in areas of demyelination[6] supported this conclusion. Electrophysiologic studies of demyelinated nerve fibers show that abnormal potassium currents contribute to conduction failure by decreasing action potential duration and amplitude.[7] Potassium channel blockers such as 4-aminopyridine (AP) and 3,4 diaminopyridine (DAP)[8] improve nerve impulse propagation in vitro, suggesting that they might be useful in treating MS patients.

Preliminary studies suggest that AP and DAP improve symptoms in some MS patients. AP improves neurologic deficits[9-14] and function[15] in MS patients, but has significant toxicity.[9,10,16] A preliminary open-label study of DAP doses up to 100 mg/day showed evidence of benefit without significant toxicity,[17] but two subsequent controlled trials using doses up to 80 mg/day in divided dosage showed little or no benefit.[16,18] We have now carried out a randomized, double-blind, placebo-controlled trial in 36 MS patients to determine the safety, tolerability, and efficacy of oral DAP in divided doses up to 100 mg/day. The primary outcome measure was improvement in prospectively defined neurologic deficits, which was leg weakness in 34 patients and arm ataxia in two. The secondary outcome measures were the patient's subjective response, results of manual motor testing of lower extremity, ratings of videotaped neurologic examinations, quadriceps and hamstrings strength

From the Departments of Neurology (Drs. Bever, Panitch, Dhib-Jalbut, Khan, and Milo, K. Conway, E. Katz, and Dr. Johnson), Physical Therapy (Dr. [illegible]on), and Epidemiology and Preventive Medicine (Dr. Hebel), School of Medicine, and the Department of Pharmaceutical Sciences (Dr. Leslie), School of Pharmacy, University of Maryland; and the Research and Neurology Services (Drs. Bever, Panitch, Dhib-Jalbut, and Khan), VA Medical Center, Baltimore, MD.

Supported by grants RG 2127-A-1 and RG 2127-B-2 from the National Multiple Sclerosis Society.

Received February 22, 1996. Accepted in final form April 23, 1996.

Address correspondence and reprint requests to Dr. Christopher T. Bever, Jr., Department of Neurology, UMH, Room N4W46, 22 South Greene St., Baltimore, MD 21201.

as measured by isometric dynamometry, neuropsychological performance, ambulation, and overall disability.

Methods. *Study medication.* 3,4 DAP was obtained from Regis Chemical Corporation (Morton Grove, IL) under an investigational new drug license to C.T.B. and formulated in capsules in the Department of Industrial Pharmacy, School of Pharmacy, University of Maryland. An active placebo was used; identical capsules were prepared containing 10 mg of nicotinic acid (a dose found in preliminary studies to produce paresthesias but not facial flushing).

DAP dosing. At the beginning of each treatment arm, patients were dose escalated from one capsule a day up to five per day (taken at 7 AM, 11 AM, 2 PM, 5 PM, and 8 PM) over a 5-day period. Patients were then maintained at that dosage unless intolerable side effects occurred, in which case patients took one-half a capsule five times a day on the same schedule.

DAP serum levels. Serum samples were drawn 30 minutes after the 11 AM dose twice during each treatment period (after 1 week on treatment and on the day of the final evaluation). Coded serum DAP levels were run using a previously published method[19] and reported to the study safety monitor (K.P.J.), who had the authority to break the blind and reduce DAP dosage if potentially dangerous DAP levels were seen. The study monitor did not break the blind on any patient or reduce dosage.

Patients. Thirty-six patients with clinical or laboratory-supported definite MS[20] between the ages of 21 and 65 were enrolled. Only patients with an acceptable study deficit were included. This was defined as a new but stable neurologic deficit or an established deficit that was worsened by heat or exercise. New but stable deficits included only deficits that had been stable for more than 2 months, but not present longer than 2 years. Patients with complicating medical illnesses were excluded as were women who were pregnant or lactating. In addition, patients with a history of seizures, unexplained syncope, or epileptiform activity on EEG were excluded. Patients who were unable to abstain from operating motor vehicles during the treatment periods were excluded. Fertile women were required to use an acceptable method of birth control. Patients were permitted to take symptomatic therapies during the trial but were required to maintain a consistent dosage and schedule. Where possible, patients were taken off baclofen during the study, and where this was not possible, dosage strengths and timing were carefully monitored and maintained constant throughout the study. The use of corticosteroids and immunosuppressive agents was not permitted during the study. The study was IRB approved, and all patients gave informed consent for participation.

Study design. Oral DAP was compared with nicotinic acid (which was selected because it produces paresthesias similar to those of DAP, but has no demonstrated effect on either MS or core body temperature). Patients were randomized to a sequence of two 30-day treatment periods separated by a 30-day washout period. Efficacy evaluations were carried out at baseline and at the end of each 30-day period by a blinded examining neurologist. Evaluations were carried out in the same facility and at the same time of day, and oral temperature was monitored to assure that differences were not due to temperature variations.

Safety evaluations. At the end of each treatment period, CBC with differential; serum chemistries including electrolytes, blood urea nitrogen, creatinine, LDH, SGOT and SGPT; coagulation profile including prothrombin time and partial thromboplastin time; and urinalysis were carried out. In addition, ECGs and EEGs were obtained.

Efficacy evaluations. Prospectively defined neurologic deficit. During the screening evaluation, the examining neurologist specified and rated the study deficit. This deficit was rated at the end of each 30-day treatment period, and at the final evaluation the examining physician indicated whether the study deficit had improved and, if so, during which treatment period it improved.

Patient subjective response. At the end of each treatment period, patients were asked whether they noted any improvement in their neurologic deficits, and their response was recorded. At the end of the second treatment period, the patients were asked which treatment had caused greater improvement.

Manual motor testing (MMT) of leg strength. Strength in the right and left iliopsoas, quadriceps, hamstrings, gastrocnemius, and anterior tibialis muscles was assessed on examination and rated using the five-point MRC scale.[21] A strength score at each time point was obtained by summing the ratings of the individual muscles.

Scored videotaped neurologic examination. The examining physician's neurologic examination was recorded at the end of each treatment period. The paired tapes from the two treatment periods were reviewed by neurologists not involved in the conduct of the trial who rated motor strength in the legs, ambulation, and overall improvement. Leg strength from videotaped motor testing (VMT) was rated in the right and left iliopsoas, quadriceps, hamstrings, gastrocnemius, and anterior tibialis muscles using the five-point MRC scale.[21] A score for each time point was obtained by summing the ratings of the individual muscles. Ambulation was rated using an arbitrary 0 to 5 scale and for the global assessment based on the evaluator's assessment as to the treatment period during which the patient appeared better neurologically.

Quadriceps and hamstrings strength (QMT) measured by isometric dynamometry. Maximum force output of the quadriceps and hamstrings muscles in isometric contraction was measured using a testing apparatus consisting of a computer-controlled hydraulically powered lever arm coupled to a force transducer (Kin-Com, Med*Ex Diagnostics, Inc., Canada). Testing was carried out at the same time of day for each patient at the same ambient temperature by the same examiner (P.A.A.). Patients were tested seated on the apparatus with 110° of hip flexion and 45° of knee extension. Strength was measured in triplicate determinations (with a 1-minute rest between replicates) of maximum isometric contractions of the quadriceps and hamstrings muscles using a Kin-Com testing apparatus. Strength was expressed in dynes/m^2.

Neuropsychological evaluation (NPT). Patients were tested using the Brief Repeatable Battery of Neuropsychological Tests for Multiple Sclerosis,[22-24] which is comprised of the Selective Reminding Test, the 10/36 Spatial Recall Test, the Symbol Digit Modalities, the Paced Auditory Serial Addition Task, and Word List Generation Tests. It was

Table 1 Summary of neurologic evaluations

Outcome measure	Number of patients improved		Mean score or power ± standard error		
	DAP	Placebo	DAP	Placebo	*p* value
Study deficit	22	2	—	—	0.0005†
Patient subjective	15	3	—	—	0.008†
Manual motor test score	17	4	41.6 ± 1.63	39.9 ± 1.7	0.002‡
Quantitative motor testing					
Hamstrings strength*	15	9	130 ± 12	123 ± 11	0.001‡
Quadriceps strength*	16	8	231 ± 27	206 ± 25	0.04‡
Video ratings					
Leg strength score	17	8	58.1 ± 2.9	56.8 ± 3.0	0.001‡
Ambulation score	11	5	4.94 ± 0.50	4.48 ± 0.49	0.054‡
Global rating	14	6	1.12 ± 0.18	0.52 ± 0.15	0.084‡
Ambulation index	5	0	5.0 ± 0.41	5.15 ± 0.45	0.02‡

* Dynes/m^2.
† Exact binomial probability.
‡ From Wilcoxon signed rank test.

administered and scored according to published procedures.[25] Tests were administered by the same examiner, at the same location, at the same time of day for all patients, and alternate forms were used for each repeated examination.

Ambulation index (AI) and Expanded Disability Status Scale (EDSS). Standard neurologic history and examination were used to score the patients on the EDSS.[26] Timed ambulation on a 25-foot course was used to rate the patients on the AI.[27]

Statistical methods. The treatment response of the prospectively defined study deficit in each patient was rated and the patient subjective response assessed at the end of the second treatment period. The significance of differences in improvement rates for the study deficit and the patient subjective response were determined using exact binomial probabilities. Paired scores (DAP treatment arm versus placebo arm within patients) from MMT, QMT, VMT, NPT, and AI were compared using the Wilcoxon signed rank test. Means and standard errors for MMT, QMT, NPT, and AI were calculated for descriptive purposes.

Results. *Patient characteristics and retention.* Thirty-six patients (14 men and 22 women) were enrolled in the study (table 1). The mean age was 44 (range, 21 to 65), mean EDSS score at entry of 6.0 (range, 2.5 to 9.0), and disease duration was 15.6 years (range, 2 to 29 years). Twenty-nine patients had chronic progressive and seven patients had relapsing-progressive MS. The study deficits in 34 were leg weakness and in 2 arm ataxia. Eight patients failed to complete the study—one because of the occurrence of a urinary tract infection with confusion and neurologic deterioration (no. 1), one for personal reasons (no. 11), one because of paresthesias and anxiety (no. 17), four because of disease progression requiring steroid treatment (nos. 25, 27, 30, and 32), and one because of the occurrence of aspiration pneumonia (no. 33). Twenty-eight patients completed the study. Thirteen received DAP during the first treatment period, and eight received it during the second. Although patients were randomly assigned to treatment order, it was found at the completion of the study that the group who received DAP first were less disabled, with an average EDSS score of 4.8 compared with an average of 7.2 in those who received DAP second.

Adverse events. Thirty-one of 36 patients reported DAP-related adverse events. The most common adverse events were paresthesias, which were reported by 25 patients on DAP and 5 patients on placebo. Abdominal pain was reported by 19 patients on DAP and only 2 on placebo. Confusion occurred in three patients on DAP and no patient on placebo; however, two of the episodes occurred in the context of complicating medical illnesses—urosepsis in patient 1 and aspiration pneumonia in patient 33. A grand mal seizure occurred in patient 4 while on DAP treatment, and no seizures occurred during the placebo arm of the trial. Dose-limiting side effects were encountered in eight patients on DAP. This was due to abdominal pain or paresthesias in seven and anxiety in one (no. 17), and was managed by reductions of DAP dosage to 10 mg five times a day in five patients and by discontinuation of treatment in three.

Efficacy. Primary outcome measure. A significant treatment-related effect was seen in the primary outcome measure, which was improvement in the prospectively defined neurologic deficit. Twenty-four patients improved—22 on DAP and 2 on placebo ($p = 0.0005$, Fisher's exact test).

Subjective response. Seventeen patients reported subjective improvement during treatment—14 improved during the DAP arm only, two improved during the placebo arm only ($p = 0.009$, Fisher's exact test), and one patient (no. 10) reported improvement during both arms.

Manual motor testing. MMT of the leg strength (see table 1) improved in 17 patients during the DAP arm and in four during the placebo arm (seven were unchanged). Mean strength scores are shown in figure 1. Patients who received DAP first are shown separately from those who

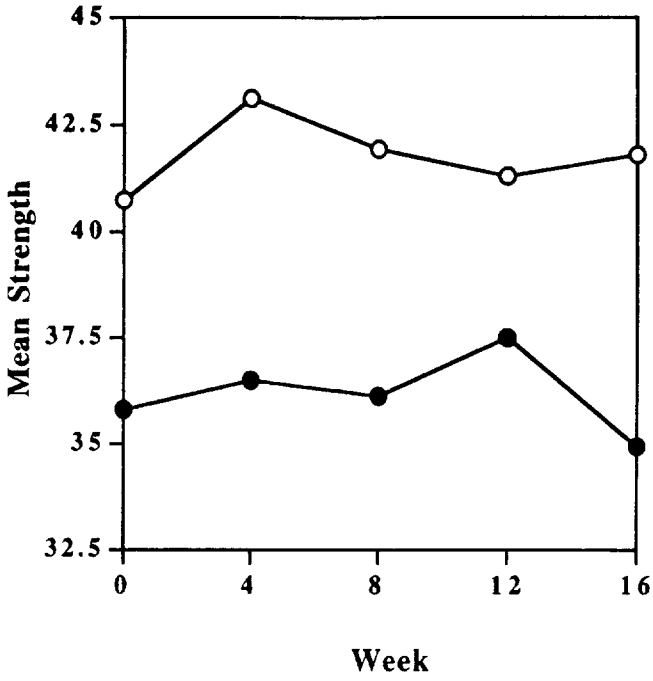


Figure 1. Graph of mean strength scores measured by manual motor testing over the 16-week trial in patients who received DAP during the first (white circles) and second (black circles) treatment periods.

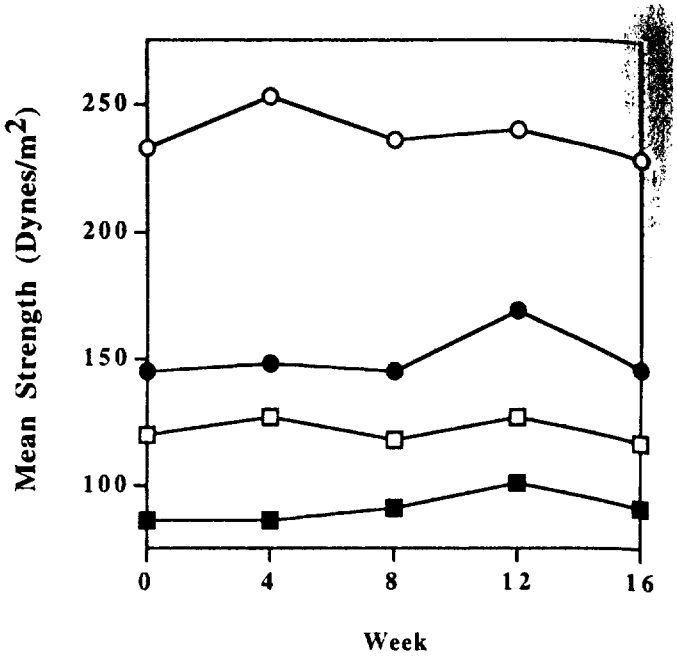


Figure 2. Graph of mean quadriceps (circles) and hamstrings (squares) strength measured by isometric dynamometry over the 16-week trial in patients who received DAP during the first (white symbols) and second (black symbols) treatment periods.

received DAP second, and because of the difference in average disability between the two groups, the baseline means are different. A second analysis was carried out comparing the scores for all patients during the DAP arm with the scores during the placebo arm. Although the mean examination score of 41.6 during the DAP treatment arm was only slightly higher than the mean score of 39.9 during the placebo arm, the difference between the two arms was statistically significant ($p = 0.002$, Wilcoxon signed rank test).

Evaluations of videotaped neurologic examinations. Videotaped neurologic examinations were available from both treatment periods on 25 patients (see table 1). Scored leg strength was significantly higher during the DAP treatment period ($p = 0.001$, Wilcoxon signed rank test). Trends in favor of DAP treatment were seen in both scored ambulation and global assessment (see table 1).

Quantitative motor testing. A significant treatment-related improvement was seen in the results of quantitative measurement of quadriceps and hamstrings strength (see table 1). Changes in mean strengths are shown in figure 2. Again, patients who received DAP first are shown separately from those who received DAP second, and the baseline means are different for the two groups. A separate analysis comparing all scores for the two treatment arms showed that mean hamstrings strength was 130 dynes/m^2 during the DAP-treatment arm compared with 124 dynes/m^2 during the placebo arm ($p = 0.001$, Wilcoxon signed rank test). Mean quadriceps strength was 233 dynes/m^2 during the DAP-treatment arm and 210 dynes/m^2 during the placebo-treatment arm ($p = 0.041$, Wilcoxon signed rank test).

Ambulation. Mean AI over the course of the trial is shown in figure 3. Again, mean baseline AIs for the two treatment groups (DAP first versus placebo first) were slightly different. In a separate analysis comparing scores during DAP treatment with those during placebo treatment, improvements in AI were seen during DAP treatment ($p = 0.022$, Wilcoxon signed rank test).

Responder analysis. A responder analysis was carried out to determine whether improvement in the study deficit correlated with improvement in MMT, VMT, and QMT. Of the 21 patients who had improvement in their study deficit

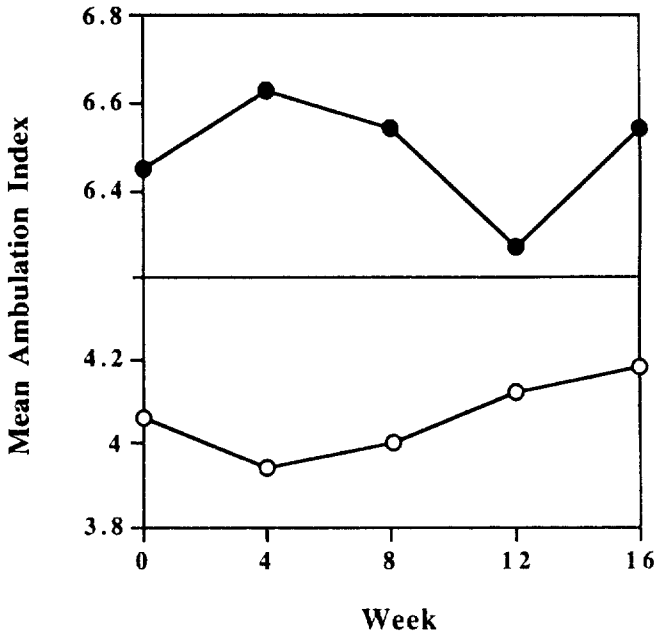


Figure 3. Graph of mean ambulation index over the 16-week trial in patients who received DAP during the first (white circles) and second (black circles) treatment periods.

Table 2 Summary of neuropsychological test results (mean score)

Outcome measure	DAP	Placebo
ctive reminding	37.5 ± 10.3	36.9 ± 12.4
10/36 spatial recall (long-term storage)	18.8 ± 5.0	17.2 ± 5.7
Symbol digit modalities	34.2 ± 15.5	34.5 ± 17.6
Paced auditory serial addition	66.6 ± 24.7	65.4 ± 24.0
Word list generation	28.6 ± 10.2	27.7 ± 9.4

(leg strength), 19 had improvement in at least two of the other measures, and 10 had improvement in all.

Other efficacy evaluations. None of the outcome measures showed evidence of a period or carry-over effect (Fisher's exact test, results not given). No significant treatment-related changes in NP performance were seen (table 2). No changes in EDSS score were seen during either treatment arm (results not given). Thirteen of the 22 patients with improvement in their prospectively defined neurologic deficits elected to enter an open-label extension of treatment.

Serum level data. The magnitude of peak serum DAP levels correlated with adverse events but not efficacy. Serum level data were available on 28 patients. DAP was detected in 26 patients during the DAP-treatment period, and no DAP was detected in the serum of any patient during the placebo-treatment period. The mean peak se- DAP level was 44 ± 7.4 ng/mL. The mean peak level e 10 patients in whom dosage reduction was necessary due to adverse events was 69 ± 19 ng/mL whereas the mean peak level in 18 patients who did not require a dosage reduction was 37.2 ± 7.3 ng/mL ($p < 0.05$, Student's t test). The mean peak level in patients who had improvement in study deficit, MMT, VMT, and QMT was 41 ± 9.1 ng/mL, not significantly different from the mean for all patients.

Discussion. Treatment with oral DAP in total daily doses up to 100 mg/day produced improvement in prospectively defined neurologic deficits in MS patients in a double-blind, placebo-controlled, crossover trial. In addition, lower-extremity strength, as measured by manual and quantitative isometric testing, and lower-extremity function, as indicated by improvement in AI, improved. These results are consistent with the results of an open label trial.[17] One previous placebo-controlled trial of DAP doses up to 80 mg/day showed subjective but not objective improvements in MS patients.[18] A second, blinded, crossover comparison of oral DAP in doses of 40 to 80 mg/day with oral AP showed improvement in neurophysiologic tests of visual function comparable with AP,[28] but no improvements in ambulation, vision, and spasticity. The only clinically relevant changes improvements in concentration in one patient fatigue in one patient of ten tested. AP produces similar motor improvements,[9] which are related to total drug exposure, not peak serum concentration. Although DAP treatment did not improve EDSS scores as AP treatment did in one trial,[15] five patients had improvement of ambulation as reflected in the AI. The present trial is the first to show significant neurologic improvements with DAP treatment in a randomized, double-blind, placebo-controlled format.

DAP doses up to 100 mg/day produced significant toxicity. Eighty-six percent of 36 patients reported side effects during the DAP arm of the trial, whereas only 20% reported them during the placebo arm. The frequency of side effects was greater in this trial than in previous trials of lower doses of DAP,[18,28] but comparable with a trial of AP in which 70% of patients reported side effects during the period of active treatment.[15] The most common side effects were paresthesias reported by 25 patients and abdominal pain reported by 19 patients during the DAP arm. These results are similar to a comparison of DAP and AP[28] and suggest that DAP has greater peripheral toxicity than AP. Abdominal pain necessitating dosage reduction occurred in six patients during the DAP arm of the present study. Studies of AP did not produce comparable results because dose titration protocols were used.[13,15] Patient no. 2, who had no history of syncope or seizures, had a generalized tonic-clonic seizure, which appeared to be DAP related. DAP[29] and AP[9,16] rarely cause seizures and are dose and serum concentration related.[9] Two serious adverse events (requiring hospitalization) occurred that were not clearly related to DAP treatment: one patient (no. 1) developed a confusional episode in the context of urosepsis while on DAP, and a second patient (no. 33), who had a history of episodes of choking with airway obstruction, had a similar episode resulting in an aspiration pneumonia while on DAP. Similar to the experience with AP,[9] DAP toxicity appears to be related to peak serum levels.[17] Because increased tolerability of AP has been achieved by the use of a controlled-release formulation,[30] and the serum half-life of DAP is shorter than AP,[17] a similar approach might be useful with DAP. Although DAP treatment appears to improve leg strength and ambulation in some MS patients, it has significant toxicity, and its use should be limited to therapeutic trials until definitive trials show that it is safe and effective.

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A double-blind placebo-controlled study of 3,4-diaminopyridine in amytrophic lateral sclerosis patients on a rehabilitation unit

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Abstract

3,4-Diaminopyridine (DAP) enhances acetylcholine release from the nerve terminal and improves conduction in demyelinated axons. In this double-blinded placebo controlled cross over study we examined the effects of DAP combined with inpatient rehabilitation in nine patients with disabling motor weakness due to amyotrophic lateral sclerosis (ALS). A single dose of DAP or placebo was increased daily to the maximum (range: 10–80 mg) tolerated dose: after patients were assessed on the first treatment, the alternate drug was given in the same manner. Functional Independence Measurement (FIM), Ashworth, grip strength, limb strength measurements, nerve conduction studies and speech assessments were initiated 1/2 h after receiving the maximum tolerated dose of DAP or placebo. DAP was tolerated in all patients, but limited by gastrointestinal side effects in four patients. The mean peak serum level was 20.11 (S.D. = 5.11) ng/ml, occurring 1.25 (S.D. = 0.56) h after dose. A statistically significant improvement in FIM and speech asessment scores between admission and discharge occurred. However, no significant differences in clinical or electrophysiologic measures were seen between DAP and placebo treatments. This study suggests that intensive inpatient rehabilitation has a role in the management of patients with ALS, but DAP does not diminish motor impairment.

Keywords: Amyotrophic lateral sclerosis; Diaminopyridine; Rehabilitation

1. Introduction

Amyotrophic lateral sclerosis (ALS) causes insidiously progressive motor weakness due to degeneration of pyramidal tracts and motor neurons. Conventional management currently focuses on treating the neurological and medical complications of the illness. Recently we reported the results of an open label study of single dose oral 3,4-diaminopyridine (DAP) combined with inpatient rehabilitation in patients with severe late stage ALS for symptomatic treatment of disabling motor weakness (Aisen et al., 1994). DAP is a slow potassium channel blocker which enhances acetylcholine release from the nerve terminal and improves conduction in unmyelinated and demyelinated nerve. The aminopyridines have shown promise in ameliorating motor weakness in other diseases of central and peripheral nervous system (Lundh et al., 1984; Bever et al., 1994 and Bever et al., 1990; McEvoy et al., 1989; Murray and Newsom-Davis, 1981).

In addition to causing degeneration on motor neurons, ALS is associated with corticospinal tract degeneration with demyelination; significant prolongation of central motor conduction latencies have been reported (Hugon et al., 1987; Ingram and Swash, 1987). Our rationale for choosing DAP as a symptomatic treatment for disabling limb paresis in ALS was based on its potential for enhancing central conduction velocity. In addition, DAP can improve peripheral synaptic efficiency, and previous studies have suggested short-term benefit in strength in ALS patients given guanidine, another drug which enhances acetylcholine release from the nerve terminal (Norris, 1973).

In the pilot study, DAP was well tolerated in patients with advanced ALS. Doses of 20–80 mg appeared to produce a modest increase in strength and a significant improvement in functional status, as measured by the Functional Independence Measure (FIM) (Granger et al., 1986). Improvements in strength and functional status were maintained 1–3 weeks after the drug was discontinued.

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To ascertain whether DAP and/or inpatient rehabilitation have a meaningful role in ALS, we conducted a double-blind placebo controlled crossover study of the effects of single oral dose therapy on motor strength, functional status and nerve conduction (NCV).

2. Materials and methods

Nine patients (5 male, 4 female; ages 47–75; 0.25–9.00 years since diagnosis) with disabling motor weakness due to advanced (ALS) were admitted to the Burke Rehabilitation Hospital. The diagnosis was based on a history of progressive weakness, clinical evidence of upper and lower motor neuron dysfunction, electromyographic evidence of denervation in a minimum of three limbs and the exclusion of other conditions. Before entering the study all patients had an electrocardiogram, an electroencephalogram, a complete blood count, and renal and liver function tests; any significant abnormality precluded study participation. The protocol was approved by the Institutional Review Board of the Burke Rehabilitation Hospital. Written informed consent was obtained.

Each patient received daily individualized physical and occupational therapy. Speech therapy was prescribed to 4/9 patients on the basis of clinical need.

Patients received a daily oral dose of the drug studied with either breakfast or lunch. The Burke Rehabilitation Hospital pharmacy compounded 250 mg lactose with 10 mg DAP in clear gelatin capsules. Placebo capsules contained only lactose. Patients received either DAP or placebo ('drug 1') during the first evaluation period, and the alternative ('drug 2') during the second. DAP or placebo was administered by the pharmacy to patients in code format. Investigators, therapists, and study subjects remained blinded to dosage contents. The code was not broken until all subjects completed the study and were discharged. Dosage started at 10 mg and increased daily to the maximum tolerated dose, which did not exceed 80 mg. Patients were assessed half an hour after receiving maximum drug dosage.

Each patient had clinical and electrophysiological evaluations at admission, within 1 h after maximum dose of drug 1, within 1 h after maximum dose of drug 2, and at discharge.

Assessments included nerve conduction tests of two motor nerves, usually median and tibial. Nerve conduction velocities, distal latencies, evoked response amplitudes and F response latencies were recorded. Pulmonary function measurements (P_iMax and P_eMax) were performed by a respiratory therapist. Functional performance was quantified by FIM scores generated by a certified occupational therapist. The FIM is an established and validated functional status instrument designed for use in the rehabilitation population (Dodds et al., 1993). The FIM requires rating the performance of 18 tasks of daily living from 1 (dependent) to 7 (independent), and calculating the sum (maximum score 126). Motor function was quantified by a neurologist, using the scale developed in the previous study (Aisen et al., 1994; Bensimon et al., 1994; Lacomblez et al., 1989). Strength in fourteen individual muscle groups was scored (0 = no contraction – 5 = normal strength) and summed. The Ashworth scale was used to assess muscle tone (Ashworth, 1964).

Serum samples were taken every half hour for up to 2 h after patients received maximum levels of drug, and DAP concentrations were measured on site with high performance liquid chromatography, after completion of the clinical trial. Statistical analysis of data was performed on a Macintosh II computer using the Statview II software program. One factor analysis of variance (ANOVA) with post hoc analysis was performed to compare admission and discharge performance and DAP and placebo performance.

3. Results

The average length of stay for the study population was 20.33 days (S.D. = 4.30 days). The mean maximum tolerated dose of DAP was 44.44 mg (S.D. = 24.68 mg; range 10 to 80 mg) and of placebo was 46.11 mg (S.D. = 23.69

Table 1
Side effects

Patient	DAP (mg)	Side effects	Severity	Placebo (mg)	Side effects	Severity
A	80	Tingling (perioral and fingertips)	Mild	80	Bad taste in mouth	Mild
B	10	Tingling (left hand)	Mild	10	None	N/A
C	60	Tingling (perioral, fingers, neck), abdominal cramping	Mild	60	Tingling (perioral)	Mild
D	60	Abdominal cramping	Mild	60	Tingling (perioral), abdominal cramping	N/A
E	30	None	N/A	30	Tingling (perioral), anxiety	Mild
F	60	None	N/A	60	Tingling (right leg)	Mild
G	60	Tingling (perioral)	Mild	60	None	N/A
H	25	Tingling (facial 30 mg), abdominal cramping	Severe	40	Abdominal cramping	Mild
I	15	Tingling (perioral 20 mg), abdominal cramping	Moderate	15	None	N/A

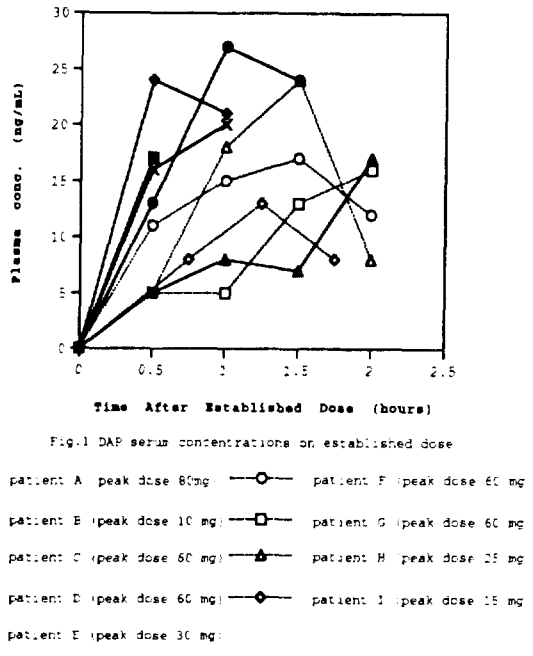

Fig. 1. DAP serum concentrations on established dose.

mg; range 10 to 80 mg). Side effects occurred on DAP and placebo as shown in Table 1, and included paresthesia, ...iety, and abdominal cramping.

Analysis of FIM scores on DAP and placebo showed no significant difference ($p = 0.902$; mean DAP score = 102.44, S.D. = 19.11, placebo = 102.67, S.D. = 19.16). However, a statistically significant improvement between admission and discharge FIM scores was evident ($p = 0.033$). The group mean score increased from 96.5 (S.D. = 18.21) on admission to 101.88 (17.84) on discharge.

Motor strength did not significantly change either on DAP ($p = 0.966$; mean DAP score = 55.53, S.D. = 11.05; placebo = 55.59, S.D. = 4.49) or between admission and discharge ($p = 0.782$; mean DAP score = 53.61, S.D. = 11.01, placebo = 53.24, S.D. = 13.52). Similarly, grip strength recordings and Ashworth assessments also showed no significant changes.

Speech intelligibility scores similarly showed no difference between DAP and placebo ($p = 0.480$; mean DAP score = 47.50, S.D. = 35.83, placebo = 51.5, S.D. = 31.26) but did improve significantly between admission and discharge ($p = 0.0486$; mean DAP admission score = 34.00, S.D. = 35.63, discharge = 50.50, S.D. = 33.20).

Nerve conduction velocities, evoked response amplitudes, and F-wave latencies showed no significant differences among admission, DAP, and placebo assessments. No reversals in conduction block occurred. There was also significant change in group P_1Max and P_2Max performance from admission to discharge and between DAP and placebo.

Peak serum levels of DAP ranged from 13–27 ng/ml. The mean peak serum level was 20.11 ng/mL (S.D. = 5.11). The average time to peak level was 1.25 h (0.56). (Fig. 1).

4. Discussion

As in our previous study, subjects treated with multidisciplinary rehabilitation and DAP experienced an improvement in functional status. This study showed improvement occurring independent of DAP or placebo treatment, and was sustained after all study drug was discontinued. Motor strength scores increased to a degree which did not achieve significance on both active medication and placebo, and declined after the drug was discontinued. These findings are consistent with our prior open label study, and suggest that changes in strength reflect a placebo effect. They also suggest that short term intensive multidisciplinary rehabilitation improves function in patients with profound impairment and disability from advanced ALS. We conclude that oral DAP does not have a useful role in the treatment of advanced ALS, but that short term inpatient rehabilitation may, despite the progressive nature of the disease. Short-term intensive inpatient rehabilitation is not conventional in this population, perhaps because of a prevailing belief that its cost is not warranted in an incurable disease. Further study is needed to clarify the degree of benefit in terms of quality of life, morbidity, mortality and economics. It is important to determine how long lasting these effects are and explore alternatives to inpatient rehabilitation in controlled pilot programs.

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A double-blind placebo-controlled study of 3,4-diaminopyridine in amytrophic lateral sclerosis patients on a rehabilitation unit

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Abstract

3,4-Diaminopyridine (DAP) enhances acetylcholine release from the nerve terminal and improves conduction in demyelinated axons. In this double-blinded placebo controlled cross over study we examined the effects of DAP combined with inpatient rehabilitation in nine patients with disabling motor weakness due to amyotrophic lateral sclerosis (ALS). A single dose of DAP or placebo was increased daily to the maximum (range: 10–80 mg) tolerated dose; after patients were assessed on the first treatment, the alternate drug was given in the same manner. Functional Independence Measurement (FIM), Ashworth, grip strength, limb strength measurements, nerve conduction studies and speech assessments were initiated 1/2 h after receiving the maximum tolerated dose of DAP or placebo. DAP was tolerated in all patients, but limited by gastrointestinal side effects in four patients. The mean peak serum level was 20.11 (S.D. = 5.11) ng/ml, occurring 1.25 (S.D. = 0.56) h after dose. A statistically significant improvement in FIM and speech asessment scores between admission discharge occurred. However, no significant differences in clinical or electrophysiologic measures were seen between DAP and placebo treatments. This study suggests that intensive inpatient rehabilitation has a role in the management of patients with ALS, but DAP does not diminish motor impairment.

Keywords: Amyotrophic lateral sclerosis; Diaminopyridine; Rehabilitation

1. Introduction

Amyotrophic lateral sclerosis (ALS) causes insidiously progressive motor weakness due to degeneration of pyramidal tracts and motor neurons. Conventional management currently focuses on treating the neurological and medical complications of the illness. Recently we reported the results of an open label study of single dose oral 3,4-diaminopyridine (DAP) combined with inpatient rehabilitation in patients with severe late stage ALS for symptomatic treatment of disabling motor weakness (Aisen et al., 1994). DAP is a slow potassium channel blocker which enhances acetylcholine release from the nerve terminal and improves conduction in unmyelinated and demyelinated nerve. The aminopyridines have shown promise in ameliorating motor weakness in other diseases of central and peripheral nervous system (Lundh et al., 1984; Bever et al., 1994 and Bever et al., 1990; McEvoy et al., 1989; Murray and Newsom-Davis, 1981).

In addition to causing degeneration on motor neurons, ALS is associated with corticospinal tract degeneration with demyelination; significant prolongation of central motor conduction latencies have been reported (Hugon et al., 1987; Ingram and Swash, 1987). Our rationale for choosing DAP as a symptomatic treatment for disabling limb paresis in ALS was based on its potential for enhancing central conduction velocity. In addition, DAP can improve peripheral synaptic efficiency, and previous studies have suggested short-term benefit in strength in ALS patients given guanidine, another drug which enhances acetylcholine release from the nerve terminal (Norris, 1973).

In the pilot study, DAP was well tolerated in patients with advanced ALS. Doses of 20–80 mg appeared to produce a modest increase in strength and a significant improvement in functional status, as measured by the Functional Independence Measure (FIM) (Granger et al., 1986). Improvements in strength and functional status were maintained 1–3 weeks after the drug was discontinued.

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To ascertain whether DAP and/or inpatient rehabilitation have a meaningful role in ALS, we conducted a double-blind placebo controlled crossover study of the effects of single oral dose therapy on motor strength, functional status and nerve conduction (NCV).

2. Materials and methods

Nine patients (5 male, 4 female; ages 47–75; 0.25–9.00 years since diagnosis) with disabling motor weakness due to advanced (ALS) were admitted to the Burke Rehabilitation Hospital. The diagnosis was based on a history of progressive weakness, clinical evidence of upper and lower motor neuron dysfunction, electromyographic evidence of denervation in a minimum of three limbs and the exclusion of other conditions. Before entering the study all patients had an electrocardiogram, an electroencephalogram, a complete blood count, and renal and liver function tests; any significant abnormality precluded study participation. The protocol was approved by the Institutional Review Board of the Burke Rehabilitation Hospital. Written informed consent was obtained.

Each patient received daily individualized physical and occupational therapy. Speech therapy was prescribed to 4/9 patients on the basis of clinical need.

Patients received a daily oral dose of the drug studied with either breakfast or lunch. The Burke Rehabilitation Hospital pharmacy compounded 250 mg lactose with 10 mg DAP in clear gelatin capsules. Placebo capsules contained only lactose. Patients received either DAP or placebo ('drug 1') during the first evaluation period, and the alternative ('drug 2') during the second. DAP or placebo was administered by the pharmacy to patients in code format. Investigators, therapists, and study subjects remained blinded to dosage contents. The code was not broken until all subjects completed the study and were discharged. Dosage started at 10 mg and increased daily to the maximum tolerated dose, which did not exceed 80 mg. Patients were assessed half an hour after receiving maximum drug dosage.

Each patient had clinical and electrophysiological evaluations at admission, within 1 h after maximum dose of drug 1, within 1 h after maximum dose of drug 2, and at discharge.

Assessments included nerve conduction tests of two motor nerves, usually median and tibial. Nerve conduction velocities, distal latencies, evoked response amplitudes and F response latencies were recorded. Pulmonary function measurements (P_iMax and P_eMax) were performed by a respiratory therapist. Functional performance was quantified by FIM scores generated by a certified occupational therapist. The FIM is an established and validated functional status instrument designed for use in the rehabilitation population (Dodds et al., 1993). The FIM requires rating the performance of 18 tasks of daily living from 1 (dependent) to 7 (independent), and calculating the sum (maximum score 126). Motor function was quantified by a neurologist, using the scale developed in the previous study (Aisen et al., 1994; Bensimon et al., 1994; Lacomblez et al., 1989). Strength in fourteen individual muscle groups was scored (0 = no contraction – 5 = normal strength) and summed. The Ashworth scale was used to assess muscle tone (Ashworth, 1964).

Serum samples were taken every half hour for up to 2 h after patients received maximum levels of drug, and DAP concentrations were measured on site with high performance liquid chromatography, after completion of the clinical trial. Statistical analysis of data was performed on a Macintosh II computer using the Statview II software program. One factor analysis of variance (ANOVA) with post hoc analysis was performed to compare admission and discharge performance and DAP and placebo performance.

3. Results

The average length of stay for the study population was 20.33 days (S.D. = 4.30 days). The mean maximum tolerated dose of DAP was 44.44 mg (S.D. = 24.68 mg; range 10 to 80 mg) and of placebo was 46.11 mg (S.D. = 23.6

Table 1
Side effects

Patient	DAP (mg)	Side effects	Severity	Placebo (mg)	Side effects	Severity
A	80	Tingling (perioral and fingertips)	Mild	80	Bad taste in mouth	Mild
B	10	Tingling (left hand)	Mild	10	None	N/A
C	60	Tingling (perioral, fingers, neck), abdominal cramping	Mild	60	Tingling (perioral)	Mild
D	60	Abdominal cramping	Mild	60	Tingling (perioral), abdominal cramping	N/A
E	30	None	N/A	30	Tingling (perioral), anxiety	Mild
F	60	None	N/A	60	Tingling (right leg)	Mild
G	60	Tingling (perioral)	Mild	60	None	N/A
H	25	Tingling (facial 30 mg), abdominal cramping	Severe	40	Abdominal cramping	Mild
I	15	Tingling (perioral 20 mg), abdominal cramping	Moderate	15	None	N/A

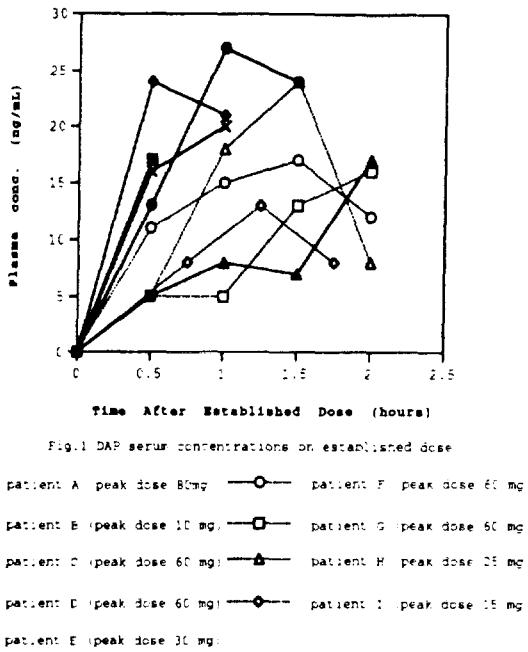

Fig. 1. DAP serum concentrations on established dose.

mg; range 10 to 80 mg). Side effects occurred on DAP and placebo as shown in Table 1, and included paresthesia, anxiety, and abdominal cramping.

Analysis of FIM scores on DAP and placebo showed no significant difference ($p = 0.902$; mean DAP score = 102.44, S.D. = 19.11, placebo = 102.67, S.D. = 19.16). However, a statistically significant improvement between admission and discharge FIM scores was evident ($p = 0.033$). The group mean score increased from 96.5 (S.D. = 18.21) on admission to 101.88 (17.84) on discharge.

Motor strength did not significantly change either on DAP ($p = 0.966$; mean DAP score = 55.53, S.D. = 11.05; placebo = 55.59, S.D. = 4.49) or between admission and discharge ($p = 0.782$; mean DAP score = 53.61, S.D. = 11.01, placebo = 53.24, S.D. = 13.52). Similarly, grip strength recordings and Ashworth assessments also showed no significant changes.

Speech intelligibility scores similarly showed no difference between DAP and placebo ($p = 0.480$; mean DAP score = 47.50, S.D. = 35.83, placebo = 51.5, S.D. = 31.26) but did improve significantly between admission and discharge ($p = 0.0486$; mean DAP admission score = 34.00, S.D. = 35.63, discharge = 50.50, S.D. = 33.20).

Nerve conduction velocities, evoked response amplitudes, and F-wave latencies showed no significant differences among admission, DAP, and placebo assessments. No reversals in conduction block occurred. There was also no significant change in group P_iMax and P_eMax performance from admission to discharge and between DAP and placebo.

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4. Discussion

As in our previous study, subjects treated with multidisciplinary rehabilitation and DAP experienced an improvement in functional status. This study showed improvement occurring independent of DAP or placebo treatment, and was sustained after all study drug was discontinued. Motor strength scores increased to a degree which did not achieve significance on both active medication and placebo, and declined after the drug was discontinued. These findings are consistent with our prior open label study, and suggest that changes in strength reflect a placebo effect. They also suggest that short term intensive multidisciplinary rehabilitation improves function in patients with profound impairment and disability from advanced ALS. We conclude that oral DAP does not have a useful role in the treatment of advanced ALS, but that short term inpatient rehabilitation may, despite the progressive nature of the disease. Short-term intensive inpatient rehabilitation is not conventional in this population, perhaps because of a prevailing belief that its cost is not warranted in an incurable disease. Further study is needed to clarify the degree of benefit in terms of quality of life, morbidity, mortality and economics. It is important to determine how long lasting these effects are and explore alternatives to inpatient rehabilitation in controlled pilot programs.

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Treatment with oral 3,4 diaminopyridine improves leg strength in multiple sclerosis patients:

Results of a randomized, double-blind, placebo-controlled, crossover trial

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Article abstract—To examine the efficacy and toxicity of oral 3,4 diaminopyridine (DAP) in dosages up to 100 mg/day, 36 patients with multiple sclerosis (MS) enrolled in a randomized, double-blind, placebo-controlled, crossover trial. The primary outcome measure was improvement of a prospectively defined neurologic deficit, which was leg weakness in 34 patients. Secondary outcome measures included the patient's subjective response, scored manual motor testing (MMT) of leg strength, scored leg strength from videotaped motor testing (VMT), quadriceps and hamstrings strength (QMT) measured by isometric dynamometry, neuropsychological testing (NPT), ambulation index (AI), and Expanded Disability Status Scale (EDSS) score. Paresthesias and abdominal pain were common and were dose limiting in eight patients. Three patients had episodes of confusion, and one patient had a seizure while on DAP. Eight patients withdrew from the study, leaving 28 evaluable patients for the efficacy analysis. The prospectively defined neurologic deficit improved in 24 patients—22 on DAP and 2 on placebo ($p = 0.0005$). All improvements were in leg weakness. Subjective response and measures of leg strength and function (MMT, VMT, QMT, and AI) improved on DAP compared with placebo. Neither NPT nor EDSS scores improved. DAP treatment can induce improvements in leg strength in MS patients, but toxicity is limiting in many patients.

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Multiple sclerosis (MS) is a primary inflammatory demyelinating disease of the CNS that frequently causes chronic neurologic symptoms[1] that vary widely from patient to patient depending on the location and extent of demyelination.[2] Although symptomatic treatments are available for some MS symptoms,[3] there are no pharmacologic treatments for leg weakness, one of the most common and disabling MS symptoms. The observations that cooling[4] and changes in serum ionized calcium[5] could cause improvement of neurologic symptoms in MS patients suggested that the dysfunction was, in part, physiologic rather than being due to axonal or neuronal loss. Pathologic studies showing relative preservation of axons in areas of demyelination[6] supported this conclusion. Electrophysiologic studies of demyelinated nerve fibers show that abnormal potassium currents contribute to conduction failure by decreasing action potential duration and amplitude.[7] Potassium channel blockers such as 4-aminopyridine (AP) and 3,4 diaminopyridine (DAP)[8] improve nerve impulse propagation in vitro, suggesting that they might be useful in treating MS patients.

Preliminary studies suggest that AP and DAP improve symptoms in some MS patients. AP improves neurologic deficits[9-14] and function[15] in MS patients, but has significant toxicity.[9,10,16] A preliminary open-label study of DAP doses up to 100 mg/day showed evidence of benefit without significant toxicity,[17] but two subsequent controlled trials using doses up to 80 mg/day in divided dosage showed little or no benefit.[16,18] We have now carried out a randomized, double-blind, placebo-controlled trial in 36 MS patients to determine the safety, tolerability, and efficacy of oral DAP in divided doses up to 100 mg/day. The primary outcome measure was improvement in prospectively defined neurologic deficits, which was leg weakness in 34 patients and arm ataxia in two. The secondary outcome measures were the patient's subjective response, results of manual motor testing of lower extremity, ratings of videotaped neurologic examinations, quadriceps and hamstrings strength

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as measured by isometric dynamometry, neuropsychological performance, ambulation, and overall disability.

Methods. *Study medication.* 3,4 DAP was obtained from Regis Chemical Corporation (Morton Grove, IL) under an investigational new drug license to C.T.B. and formulated in capsules in the Department of Industrial Pharmacy, School of Pharmacy, University of Maryland. An active placebo was used; identical capsules were prepared containing 10 mg of nicotinic acid (a dose found in preliminary studies to produce paresthesias but not facial flushing).

DAP dosing. At the beginning of each treatment arm, patients were dose escalated from one capsule a day up to five per day (taken at 7 AM, 11 AM, 2 PM, 5 PM, and 8 PM) over a 5-day period. Patients were then maintained at that dosage unless intolerable side effects occurred, in which case patients took one-half a capsule five times a day on the same schedule.

DAP serum levels. Serum samples were drawn 30 minutes after the 11 AM dose twice during each treatment period (after 1 week on treatment and on the day of the final evaluation). Coded serum DAP levels were run using a previously published method[19] and reported to the study safety monitor (K.P.J.), who had the authority to break the blind and reduce DAP dosage if potentially dangerous DAP levels were seen. The study monitor did not break the blind on any patient or reduce dosage.

Patients. Thirty-six patients with clinical or laboratory-supported definite MS[20] between the ages of 21 and 65 were enrolled. Only patients with an acceptable study deficit were included. This was defined as a new but stable neurologic deficit or an established deficit that was worsened by heat or exercise. New but stable deficits included only deficits that had been stable for more than 2 months, but not present longer than 2 years. Patients with complicating medical illnesses were excluded as were women who were pregnant or lactating. In addition, patients with a history of seizures, unexplained syncope, or epileptiform activity on EEG were excluded. Patients who were unable to abstain from operating motor vehicles during the treatment periods were excluded. Fertile women were required to use an acceptable method of birth control. Patients were permitted to take symptomatic therapies during the trial but were required to maintain a consistent dosage and schedule. Where possible, patients were taken off baclofen during the study, and where this was not possible, dosage strengths and timing were carefully monitored and maintained constant throughout the study. The use of corticosteroids and immunosuppressive agents was not permitted during the study. The study was IRB approved, and all patients gave informed consent for participation.

Study design. Oral DAP was compared with nicotinic acid (which was selected because it produces paresthesias similar to those of DAP, but has no demonstrated effect on either MS or core body temperature). Patients were randomized to a sequence of two 30-day treatment periods separated by a 30-day washout period. Efficacy evaluations were carried out at baseline and at the end of each 30-day period by a blinded examining neurologist. Evaluations were carried out in the same facility and at the same time of day, and oral temperature was monitored to assure that differences were not due to temperature variations.

Safety evaluations. At the end of each treatment period, CBC with differential; serum chemistries including electrolytes, blood urea nitrogen, creatinine, LDH, SGOT, and SGPT; coagulation profile including prothrombin time and partial thromboplastin time; and urinalysis were carried out. In addition, ECGs and EEGs were obtained.

Efficacy evaluations. Prospectively defined neurologic deficit. During the screening evaluation, the examining neurologist specified and rated the study deficit. This deficit was rated at the end of each 30-day treatment period, and at the final evaluation the examining physician indicated whether the study deficit had improved and, if so, during which treatment period it improved.

Patient subjective response. At the end of each treatment period, patients were asked whether they noted any improvement in their neurologic deficits, and their response was recorded. At the end of the second treatment period, the patients were asked which treatment had caused greater improvement.

Manual motor testing (MMT) of leg strength. Strength in the right and left iliopsoas, quadriceps, hamstrings, gastrocnemius, and anterior tibialis muscles was assessed on examination and rated using the five-point MRC scale.[21] A strength score at each time point was obtained by summing the ratings of the individual muscles.

Scored videotaped neurologic examination. The examining physician's neurologic examination was recorded at the end of each treatment period. The paired tapes from the two treatment periods were reviewed by neurologists not involved in the conduct of the trial who rated motor strength in the legs, ambulation, and overall improvement. Leg strength from videotaped motor testing (VMT) was rated in the right and left iliopsoas, quadriceps, hamstrings, gastrocnemius, and anterior tibialis muscles using the five-point MRC scale.[21] A score for each time point was obtained by summing the ratings of the individual muscles. Ambulation was rated using an arbitrary 0 to 5 scale and for the global assessment based on the evaluator's assessment as to the treatment period during which the patient appeared better neurologically.

Quadriceps and hamstrings strength (QMT) measured by isometric dynamometry. Maximum force output of the quadriceps and hamstrings muscles in isometric contraction was measured using a testing apparatus consisting of a computer-controlled hydraulically powered lever arm coupled to a force transducer (Kin-Com, Med*Ex Diagnostics, Inc., Canada). Testing was carried out at the same time of day for each patient at the same ambient temperature by the same examiner (P.A.A.). Patients were tested seated on the apparatus with 110° of hip flexion and 45° of knee extension. Strength was measured in triplicate determinations (with a 1-minute rest between replicates) of maximum isometric contractions of the quadriceps and hamstrings muscles using a Kin-Com testing apparatus. Strength was expressed in dynes/m^2.

Neuropsychological evaluation (NPT). Patients were tested using the Brief Repeatable Battery of Neuropsychological Tests for Multiple Sclerosis,[22-24] which is comprised of the Selective Reminding Test, the 10/36 Spatial Recall Test, the Symbol Digit Modalities, the Paced Auditory Serial Addition Task, and Word List Generation Tests. It was

Table 1 Summary of neurologic evaluations

Outcome measure	Number of patients improved		Mean score or power ± standard error		
	DAP	Placebo	DAP	Placebo	*p* value
Study deficit	22	2	—	—	0.0005†
Patient subjective	15	3	—	—	0.008†
Manual motor test score	17	4	41.6 ± 1.63	39.9 ± 1.7	0.002‡
Quantitative motor testing					
Hamstrings strength*	15	9	130 ± 12	123 ± 11	0.001‡
Quadriceps strength*	16	8	231 ± 27	206 ± 25	0.04‡
Video ratings					
Leg strength score	17	8	58.1 ± 2.9	56.8 ± 3.0	0.001‡
Ambulation score	11	5	4.94 ± 0.50	4.48 ± 0.49	0.054‡
Global rating	14	6	1.12 ± 0.18	0.52 ± 0.15	0.084‡
Ambulation index	5	0	5.0 ± 0.41	5.15 ± 0.45	0.02‡

* Dynes/m^2.
† Exact binomial probability.
‡ From Wilcoxon signed rank test.

administered and scored according to published procedures.[25] Tests were administered by the same examiner, at the same location, at the same time of day for all patients, and alternate forms were used for each repeated examination.

Ambulation index (AI) and Expanded Disability Status Scale (EDSS). Standard neurologic history and examination were used to score the patients on the EDSS.[26] Timed ambulation on a 25-foot course was used to rate the patients on the AI.[27]

Statistical methods. The treatment response of the prospectively defined study deficit in each patient was rated and the patient subjective response assessed at the end of the second treatment period. The significance of differences in improvement rates for the study deficit and the patient subjective response were determined using exact binomial probabilities. Paired scores (DAP treatment arm versus placebo arm within patients) from MMT, QMT, VMT, NPT, and AI were compared using the Wilcoxon signed rank test. Means and standard errors for MMT, QMT, NPT, and AI were calculated for descriptive purposes.

Results. *Patient characteristics and retention.* Thirty-six patients (14 men and 22 women) were enrolled in the study (table 1). The mean age was 44 (range, 21 to 65), mean EDSS score at entry of 6.0 (range, 2.5 to 9.0), and disease duration was 15.6 years (range, 2 to 29 years). Twenty-nine patients had chronic progressive and seven patients had relapsing-progressive MS. The study deficits in 34 were leg weakness and in 2 arm ataxia. Eight patients failed to complete the study—one because of the occurrence of a urinary tract infection with confusion and neurologic deterioration (no. 1), one for personal reasons (no. 11), one because of paresthesias and anxiety (no. 17), four because of disease progression requiring steroid treatment (nos. 25, 27, 30, and 32), and one because of the occurrence of aspiration pneumonia (no. 33). Twenty-eight patients completed the study. Thirteen received DAP during the first treatment period, and eight received it during the second. Although patients were randomly assigned to treatment order, it was found at the completion of the study that the group who received DAP first were less disabled, with an average EDSS score of 4.8 compared with an average of 7.2 in those who received DAP second.

Adverse events. Thirty-one of 36 patients reported DAP-related adverse events. The most common adverse events were paresthesias, which were reported by 25 patients on DAP and 5 patients on placebo. Abdominal pain was reported by 19 patients on DAP and only 2 on placebo. Confusion occurred in three patients on DAP and no patient on placebo; however, two of the episodes occurred in the context of complicating medical illnesses—urosepsis in patient 1 and aspiration pneumonia in patient 33. A grand mal seizure occurred in patient 4 while on DAP treatment, and no seizures occurred during the placebo arm of the trial. Dose-limiting side effects were encountered in eight patients on DAP. This was due to abdominal pain or paresthesias in seven and anxiety in one (no. 17), and was managed by reductions of DAP dosage to 10 mg five times a day in five patients and by discontinuation of treatment in three.

Efficacy. Primary outcome measure. A significant treatment-related effect was seen in the primary outcome measure, which was improvement in the prospectively defined neurologic deficit. Twenty-four patients improved—22 on DAP and 2 on placebo ($p = 0.0005$, Fisher's exact test).

Subjective response. Seventeen patients reported subjective improvement during treatment—14 improved during the DAP arm only, two improved during the placebo arm only ($p = 0.009$, Fisher's exact test), and one patient (no. 10) reported improvement during both arms.

Manual motor testing. MMT of the leg strength (see table 1) improved in 17 patients during the DAP arm and in four during the placebo arm (seven were unchanged). Mean strength scores are shown in figure 1. Patients who received DAP first are shown separately from those who

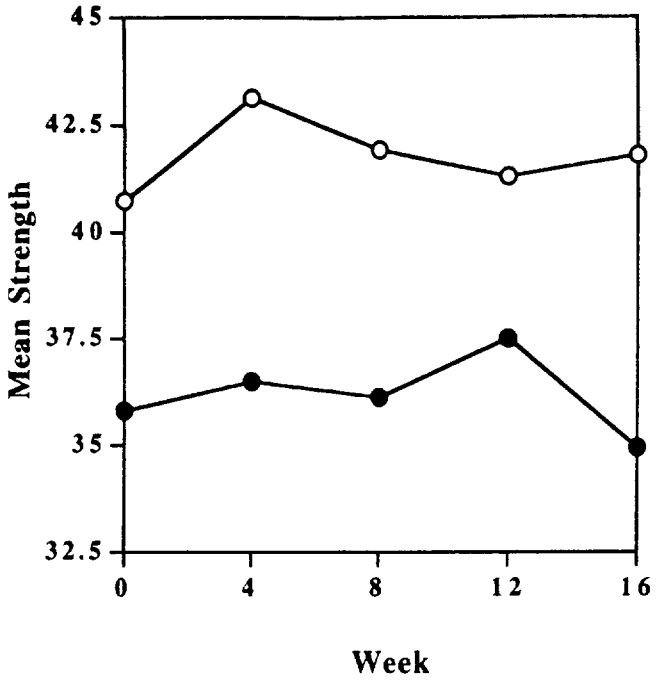

Figure 1. Graph of mean strength scores measured by manual motor testing over the 16-week trial in patients who received DAP during the first (white circles) and second (black circles) treatment periods.

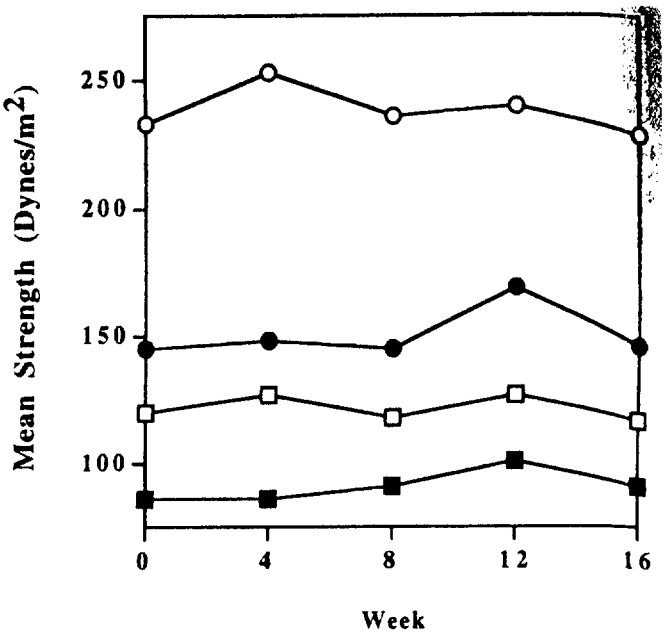

Figure 2. Graph of mean quadriceps (circles) and hamstrings (squares) strength measured by isometric dynamometry over the 16-week trial in patients who received DAP during the first (white symbols) and second (black symbols) treatment periods.

received DAP second, and because of the difference in average disability between the two groups, the baseline means are different. A second analysis was carried out comparing the scores for all patients during the DAP arm with the scores during the placebo arm. Although the mean examination score of 41.6 during the DAP treatment arm was only slightly higher than the mean score of 39.9 during the placebo arm, the difference between the two arms was statistically significant ($p = 0.002$, Wilcoxon signed rank test).

Evaluations of videotaped neurologic examinations. Videotaped neurologic examinations were available from both treatment periods on 25 patients (see table 1). Scored leg strength was significantly higher during the DAP treatment period ($p = 0.001$, Wilcoxon signed rank test). Trends in favor of DAP treatment were seen in both scored ambulation and global assessment (see table 1).

Quantitative motor testing. A significant treatment-related improvement was seen in the results of quantitative measurement of quadriceps and hamstrings strength (see table 1). Changes in mean strengths are shown in figure 2. Again, patients who received DAP first are shown separately from those who received DAP second, and the baseline means are different for the two groups. A separate analysis comparing all scores for the two treatment arms showed that mean hamstrings strength was 130 dynes/m^2 during the DAP-treatment arm compared with 124 dynes/m^2 during the placebo arm ($p = 0.001$, Wilcoxon signed rank test). Mean quadriceps strength was 233 dynes/m^2 during the DAP-treatment arm and 210 dynes/m^2 during the placebo-treatment arm ($p = 0.041$, Wilcoxon signed rank test).

Ambulation. Mean AI over the course of the trial is shown in figure 3. Again, mean baseline AIs for the two treatment groups (DAP first versus placebo first) were slightly different. In a separate analysis comparing scores during DAP treatment with those during placebo treatment, improvements in AI were seen during DAP treatment ($p = 0.022$, Wilcoxon signed rank test).

Responder analysis. A responder analysis was carried out to determine whether improvement in the study deficit correlated with improvement in MMT, VMT, and QMT. Of the 21 patients who had improvement in their study deficit

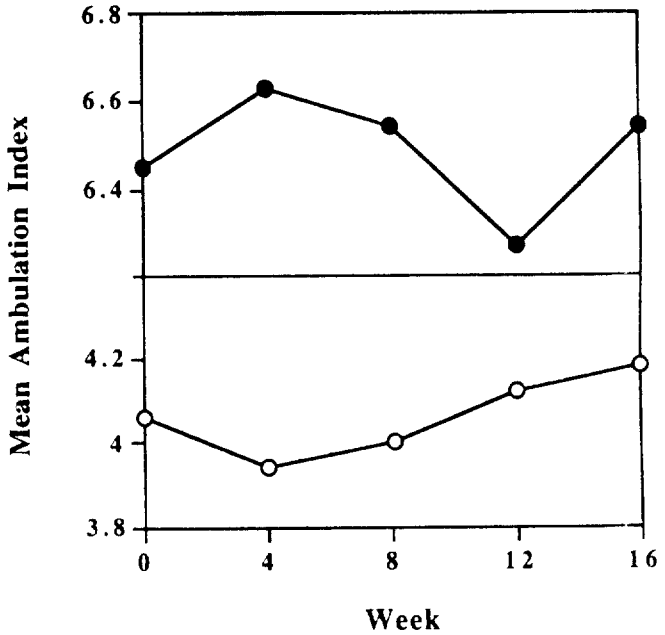

Figure 3. Graph of mean ambulation index over the 16-week trial in patients who received DAP during the first (white circles) and second (black circles) treatment periods.

Table 2 Summary of neuropsychological test results (mean score)

Outcome measure	DAP	Placebo
ctive reminding	37.5 ± 10.3	36.9 ± 12.4
36 spatial recall (long-term storage)	18.8 ± 5.0	17.2 ± 5.7
Symbol digit modalities	34.2 ± 15.5	34.5 ± 17.6
Paced auditory serial addition	66.6 ± 24.7	65.4 ± 24.0
Word list generation	28.6 ± 10.2	27.7 ± 9.4

(leg strength), 19 had improvement in at least two of the other measures, and 10 had improvement in all.

Other efficacy evaluations. None of the outcome measures showed evidence of a period or carry-over effect (Fisher's exact test, results not given). No significant treatment-related changes in NP performance were seen (table 2). No changes in EDSS score were seen during either treatment arm (results not given). Thirteen of the 22 patients with improvement in their prospectively defined neurologic deficits elected to enter an open-label extension of treatment.

Serum level data. The magnitude of peak serum DAP levels correlated with adverse events but not efficacy. Serum level data were available on 28 patients. DAP was detected in 26 patients during the DAP-treatment period, and no DAP was detected in the serum of any patient during the placebo-treatment period. The mean peak se- DAP level was 44 ± 7.4 ng/mL. The mean peak level e 10 patients in whom dosage reduction was necessary to adverse events was 69 ± 19 ng/mL whereas the mean peak level in 18 patients who did not require a dosage reduction was 37.2 ± 7.3 ng/mL ($p < 0.05$, Student's t test). The mean peak level in patients who had improvement in study deficit, MMT, VMT, and QMT was 41 ± 9.1 ng/mL, not significantly different from the mean for all patients.

Discussion. Treatment with oral DAP in total daily doses up to 100 mg/day produced improvement in prospectively defined neurologic deficits in MS patients in a double-blind, placebo-controlled, crossover trial. In addition, lower-extremity strength, as measured by manual and quantitative isometric testing, and lower-extremity function, as indicated by improvement in AI, improved. These results are consistent with the results of an open label trial.[17] One previous placebo-controlled trial of DAP doses up to 80 mg/day showed subjective but not objective improvements in MS patients.[18] A second, blinded, crossover comparison of oral DAP in doses of 40 to 80 mg/day with oral AP showed improvement in neurophysiologic tests of visual function comparable with AP,[28] but no improvements in ambulation, vision, and spasticity. The only clinically relevant changes improvements in concentration in one patient fatigue in one patient of ten tested. AP produces similar motor improvements,[9] which are related to total drug exposure, not peak serum concentration. Although DAP treatment did not improve EDSS scores as AP treatment did in one trial,[15] five patients had improvement of ambulation as reflected in the AI. The present trial is the first to show significant neurologic improvements with DAP treatment in a randomized, double-blind, placebo-controlled format.

DAP doses up to 100 mg/day produced significant toxicity. Eighty-six percent of 36 patients reported side effects during the DAP arm of the trial, whereas only 20% reported them during the placebo arm. The frequency of side effects was greater in this trial than in previous trials of lower doses of DAP,[18,28] but comparable with a trial of AP in which 70% of patients reported side effects during the period of active treatment.[15] The most common side effects were paresthesias reported by 25 patients and abdominal pain reported by 19 patients during the DAP arm. These results are similar to a comparison of DAP and AP[28] and suggest that DAP has greater peripheral toxicity than AP. Abdominal pain necessitating dosage reduction occurred in six patients during the DAP arm of the present study. Studies of AP did not produce comparable results because dose titration protocols were used.[13,15] Patient no. 2, who had no history of syncope or seizures, had a generalized tonic-clonic seizure, which appeared to be DAP related. DAP[29] and AP[9,16] rarely cause seizures and are dose and serum concentration related.[9] Two serious adverse events (requiring hospitalization) occurred that were not clearly related to DAP treatment: one patient (no. 1) developed a confusional episode in the context of urosepsis while on DAP, and a second patient (no. 33), who had a history of episodes of choking with airway obstruction, had a similar episode resulting in an aspiration pneumonia while on DAP. Similar to the experience with AP,[9] DAP toxicity appears to be related to peak serum levels.[17] Because increased tolerability of AP has been achieved by the use of a controlled-release formulation,[30] and the serum half-life of DAP is shorter than AP,[17] a similar approach might be useful with DAP. Although DAP treatment appears to improve leg strength and ambulation in some MS patients, it has significant toxicity, and its use should be limited to therapeutic trials until definitive trials show that it is safe and effective.

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A. INGREDIENT NAME:

DILOXANIDE FUROATE

B. Chemical Name:

Entamide 2-Furoate, Furamide, Furamide (Amebicide), 2-Furancarboxylic Acid, 4-((Dichloroacetyl) Methylamino) Phenyl Ester, 4-(N-Methyl-2,2-Dichloroacetamido)phenyl 2-furoate

C. Common Name:

Dichlofurazol, Diclofurazol, Histomibal, Miforon, Furentomin, Furamide, Furamid, Entamizole

D. Chemical grade or description of the strength, quality, and purity of the ingredient:

Assay 99.96%

E. Information about how the ingredient is supplied:

White Crystalline Powder, Odorless, Tasteless

F. Information about recognition of the substance in foreign pharmacopeias:

BP 1993

G. Bibliography of available safety and efficacy data including peer reviewed medical literature:

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Wolfe, M. S. Nondysenteric intestinal amebiasis. Treatment with diloxanide furoate. *JAMA,* 1973; 224(13):1601-1604.

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H. Information about dosage forms used:

Tablet

I. Information about strength:

500mg 3 times daily for 5 days or 20mg/kg/daily divided into 3 daily doses for 10 days.

J. Information about route of administration:

Orally

K. Stability data:

Melting point 114C to 116C
Stable (Hazardous Polymerization will not occur)

L. Formulations:

M. Miscellaneous Information:

30-2886
38719

CERTIFICATE OF ANALYSIS

DILOXANIDE FUROATE B.P.

BATCH # E-186/95

MFG.DATE: 08/12/1995 **EXPO. DATE:07/12/2000** **QUANTITY: 5 KG**

Description:	**White Crystalline Powder, Odorless, Tasteless.**
Solubility	**Passed**
Identification	**A) Positive** **B) Positive** **C) Positive**
Melting Range	**114° to 116°**
Free Acidity	**Passed**
Related Substances	**Complies**
Loss on drying	**0.34 %**
Sulphated Ash	**0.038 %**
Assay	**99.96 %**

QUALITY CONTROL REPORT

CHEMICAL NAME.: DILOXANIDE FUROATE A _____

MANUFACTURE LOT NO.: E-186/95

PHYSICAL TEST

SPECIFICATION TEST STANDARD.: USP__/BP__/MERCK__/NF__/MART.__/CO.SPECS.__.

1) DESCRIPTION.:
WHITE POWDER, ODORLESS.

2) SOLUBILITY.:
VERY SLIGHTLY SOLUBLE IN WATER AND ETHANOL; SOLUBLE IN CHLOROFORM.

3) MELTING POINT.:
114 C TO 116 C. K

4) SPECIFIC GRAVITY.:

5) IDENTIFICATION.:

PASSES.: _____ **FAILS.:** _____

COMMENTS.:

ANALYST SIGNATURE.: _____ **DATE.:** _____

PREPACK TEST.: _____ **DATE.:** _____ **INITIAL.:** _____

RETEST.: _____ **DATE.:** _____ **INITIAL.:** _____

---------- IDENTIFICATION ----------

PRODUCT #: D6413 NAME: DILOXANIDE FUROATE

CAS #: 3736-81-0

MF: C14H11CL2NO4

SYNONYMS

AMEBIAZOL * 8073 CB * DICHLOFURAZOL * DICLOFURAZOL * DILOXANIDE FUROATE * DILOXANID FUROATE * ENTAMIDE FUROATE * ENTAMIDE 2-FUROATE *

FURAMIDE * FURAMIDE (AMEBICIDE) * 2-FURANCARBOXYLIC ACID, 4-((DICHLOROACETYL)METHYLAMINO)PHENYL ESTER * FURENTOMIN * HISTOMIBAL * MIFORON *

---------- TOXICITY HAZARDS ----------

RTECS NO: LV1821800

2-FUROIC ACID, ESTER WITH 2,2-DICHLORO-4'-HYDROXY-N-METHYLACETANILIDE

ONLY SELECTED REGISTRY OF TOXIC EFFECTS OF CHEMICAL SUBSTANCES (RTECS)

DATA IS PRESENTED HERE. SEE ACTUAL ENTRY IN RTECS FOR COMPLETE INFORMATION.

---------- HEALTH HAZARD DATA ----------

ACUTE EFFECTS

HARMFUL IF SWALLOWED.

MAY BE HARMFUL IF INHALED.

MAY BE HARMFUL IF ABSORBED THROUGH THE SKIN.

MAY CAUSE IRRITATION.

TARGET ORGAN(S):

G.I. SYSTEM

THE TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED.

FIRST AID

IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS.

CALL A PHYSICIAN.

IN CASE OF SKIN CONTACT, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. REMOVE CONTAMINATED CLOTHING AND SHOES. CALL A PHYSICIAN.

IF INHALED, REMOVE TO FRESH AIR. IF BREATHING BECOMES DIFFICULT, CALL A PHYSICIAN.

IN CASE OF CONTACT WITH EYES, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. ASSURE ADEQUATE FLUSHING BY SEPARATING

THE EYELIDS WITH FINGERS. CALL A PHYSICIAN.

---------- PHYSICAL DATA ----------

APPEARANCE AND ODOR

SOLID.

---------- FIRE AND EXPLOSION HAZARD DATA ----------

EXTINGUISHING MEDIA

WATER SPRAY.

CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM.

SPECIAL FIREFIGHTING PROCEDURES

WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO

PREVENT CONTACT WITH SKIN AND EYES.

---------- REACTIVITY DATA ----------

STABILITY

STABLE.

HAZARDOUS POLYMERIZATION

WILL NOT OCCUR.

---------- SPILL OR LEAK PROCEDURES ----------

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED

WEAR RESPIRATOR, CHEMICAL SAFETY GOGGLES, RUBBER BOOTS AND HEAVY

RUBBER GLOVES.

SWEEP UP, PLACE IN A BAG AND HOLD FOR WASTE DISPOSAL.

AVOID RAISING DUST.

VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE.

WASTE DISPOSAL METHOD

DISSOLVE OR MIX THE MATERIAL WITH A COMBUSTIBLE SOLVENT AND BURN IN A

CHEMICAL INCINERATOR EQUIPPED WITH AN AFTERBURNER AND SCRUBBER.

OBSERVE ALL FEDERAL, STATE, AND LOCAL LAWS.

--- PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE ---

WEAR APPROPRIATE NIOSH/MSHA-APPROVED RESPIRATOR, CHEMICAL-RESISTANT

GLOVES, SAFETY GOGGLES, OTHER PROTECTIVE CLOTHING.

MECHANICAL EXHAUST REQUIRED.

HARMFUL IF SWALLOWED.

WEAR SUITABLE PROTECTIVE CLOTHING.

TARGET ORGAN(S):

G.I. SYSTEM

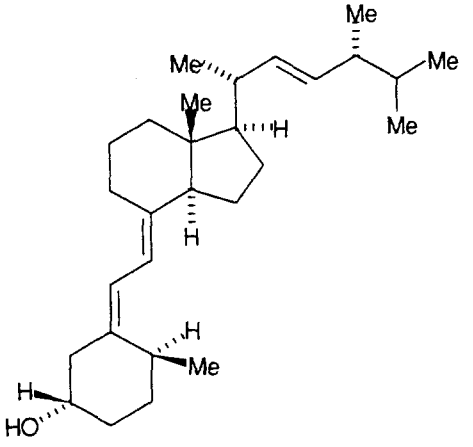
NOT BE
HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR FROM CONTACT WITH THE
ABOVE PRODUCT. SEE REVERSE SIDE OF INVOICE OR PACKING SLIP FOR ADDITIONAL
TERMS AND CONDITIONS OF SALE

dihydroergotamine tartrate EPCRS in place of the substance being examined.

Storage Dihydroergotamine Tartrate should be kept in a well-closed container and protected from light.

Action and use Used in treatment of migraine.

Dihydrotachysterol



$C_{28}H_{46}O$ 398.7 *67-96-9*

Definition Dihydrotachysterol is (5*Z*,7*E*)-(3*S*,10*S*)-9,10-secoergosta-5,7,22-trien-3-ol.

Characteristics Colourless crystals or a white, crystalline powder; odourless or almost odourless.

Practically insoluble in *water*; very soluble in *chloroform*; freely soluble in *ether*; soluble in *ethanol (96%)*; sparingly soluble in *arachis oil*.

Identification A. The *light absorption*, Appendix II B, in the range 230 to 350 nm of a 0.001% w/v solution in *methanol* exhibits three maxima, at 242, 251 and 261 nm. The *absorbance* at the maxima are 0.87, about 1.0 and about 0.65 respectively.

B. To 5 mg add 2 ml of *antimony trichloride solution* and warm in a water bath. A red colour is produced.

Melting point 126° to 129°, Appendix V A. It may also occur in a form melting at about 113°.

Specific optical rotation In a freshly prepared 2% w/v solution in *absolute ethanol*, +100° to +103°, calculated with reference to the dried substance, Appendix V F.

Tachysterol *Absorbance* of a 0.01% w/v solution in *methanol* at 280 nm, not more than 0.08, calculated with reference to the dried substance, Appendix II B.

Loss on drying When dried over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours, loses not more than 0.2% of its weight. Use 1 g.

Sulphated ash Not more than 0.1%, Appendix IX A.

Storage Dihydrotachysterol should be kept in an atmosphere of nitrogen, protected from light and stored at a temperature not exceeding 15°.

Action and use Used in treatment of hypocalcaemia.

Dill Oil

BP-1993

Definition Dill Oil is obtained by distillation from the dried ripe fruits of *Anethum graveolens* L.

Characteristics A clear, colourless or pale yellow liquid, visibly free from water; odour, characteristic of the crushed fruit.

Optical rotation +70° to +80°, Appendix V F.

Refractive index 1.481 to 1.492, Appendix V E.

Solubility in ethanol Soluble, at 20°, in 1 volume or more of *ethanol (90%)* and in 10 volumes or more of *ethanol (80%)*, Appendix X M.

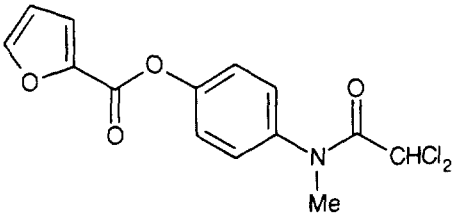
Weight per ml 0.895 to 0.910 g, Appendix V G.

Content of carvone 43.0 to 63.0% w/w, Appendix X L.

Storage Dill Oil should be kept in a well-filled, well-closed container, protected from light and stored at a temperature not exceeding 25°. It darkens in colour on storage.

Action and use Carminative.

Diloxanide Furoate



$C_{14}H_{11}Cl_2NO_4$ 328.2 *3736-81-0*

Definition Diloxanide Furoate is 4-(*N*-methyl-2,2-dichloroacetamido)phenyl 2-furoate. It contains not less than 98.0% and not more than 102.0% of $C_{14}H_{11}Cl_2NO_4$, calculated with reference to the dried substance.

Characteristics A white or almost white, crystalline powder; odourless or almost odourless.

Very slightly soluble in *water*; freely soluble in *chloroform*; slightly soluble in *ethanol (96%)* and in *ether*.

Identification A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of diloxanide furoate.

B. The *light absorption*, Appendix II B, in the range 240 to 350 nm of a 0.0014% w/v solution in *ethanol (96%)* exhibits a maximum only at 258 nm. The *absorbance* at the maximum is about 0.98.

C. Burn 20 mg by the method for *oxygen-flask combustion*, Appendix VIII C, using 10 ml of 1M *sodium hydroxide* as the absorbing liquid. When the process is complete, acidify the liquid with *nitric acid* and add *silver nitrate solution*. A white precipitate is produced.

Melting point 114° to 116°, Appendix V A.

Free acidity Shake 3 g with 50 ml of *water*, filter and wash the residue with three 20-ml quantities of *water*. Titrate the combined filtrate and washings with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Not more than 1.3 ml is required.

Related substances Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* HF_{254} as

the coating substance and a mixture of 96 volumes of *dichloromethane* and 4 volumes of *methanol* as the mobile phase. Apply separately to the plate 5 µl of each of two solutions of the substance being examined in *chloroform* containing (1) 10.0% w/v and (2) 0.025% w/v. After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2).

Loss on drying When dried to constant weight at 105°, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash Not more than 0.1%, Appendix IX A.

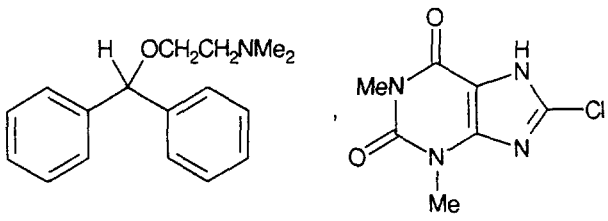
Assay Dissolve 0.3 g in 50 ml of *anhydrous pyridine* and carry out Method II for *non-aqueous titration*, Appendix VIII A, using 0.1M *tetrabutylammonium hydroxide VS* as titrant and determining the end point potentiometrically. Each ml of 0.1M *tetrabutylammonium hydroxide VS* is equivalent to 32.82 mg of $C_{14}H_{11}Cl_2NO_4$.

Storage Diloxanide Furoate should be protected from light.

Preparation
Diloxanide Tablets

Action and use Antiprotozoal.

Dimenhydrinate ☆



$C_{17}H_{21}NO,C_7H_7ClN_4O_2$ 470.0 *523-87-5*

Definition Dimenhydrinate contains not less than 53.0% and not more than 55.5% of diphenhydramine (2-benzhydryloxyethyldimethylamine, $C_{17}H_{21}NO$; 255.4) and not less than 44.0% and not more than 46.5% of 8-chlorotheophylline (8-chloro-1,3-dimethylpurine-2,6(3*H*,1*H*)-dione, $C_7H_7ClN_4O_2$; 214.6), both calculated with reference to the dried substance.

Characteristics Colourless crystals or a white, crystalline powder.

Slightly soluble in *water*; freely soluble in *chloroform* and in *ethanol (96%)*; sparingly soluble in *ether*.

Identification *Test C may be omitted if tests A, B and D are carried out. Tests A, B and D may be omitted if test C is carried out.*

A. *Melting point*, 102° to 106°, Appendix V A, Method I.

B. Dissolve 0.1 g in a mixture of 3 ml of *water* and 3 ml of *ethanol (96%)*, add 6 ml of *water* and 1 ml of 2M *hydrochloric acid* and cool in ice for 30 minutes, scratching the side of the tube with a glass rod, if necessary, to initiate crystallisation. Dissolve about 10 mg of the precipitate in 1 ml of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness in a porcelain dish. A reddish residue remains, which becomes violet-red when exposed to ammonia vapour.

C. The *infrared absorption spectrum*, Appendix II A, is concordant with the spectrum of *dimenhydrinate EPCRS*.

D. Dissolve 0.2 g in 10 ml of *ethanol (96%)*, add 10 ml of *picric acid solution* and initiate crystallisation by scratching the side of the tube with a glass rod. The *melting point* of the precipitate, after washing with *water* and drying at 100° to 105°, is 130° to 134°, Appendix V A, Method I.

Alkalinity To 0.4 g add 20 ml of *carbon dioxide-free water*, shake for 2 minutes and filter. The pH of the filtrate is 7.1 to 7.6, Appendix V L.

Clarity and colour of solution A 5.0% w/v solution in *ethanol (96%)* is *clear*, Appendix IV A, and *colourless*, Appendix IV B, Method II.

Heavy metals A 10% w/v solution in a mixture of 85 volumes of *acetone* and 15 volumes of *water* complies with *limit test B for heavy metals*, Appendix VII. Prepare the standard using a lead standard solution (2 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb)* with the acetone—water mixture (20 ppm).

Theophylline and substances related to diphenhydramine Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel* GF_{254} as the coating substance and a mixture of 90 volumes of *dichloromethane*, 9 volumes of *methanol* and 1 volume of 13.5M *ammonia* as the mobile phase. Apply separately to the plate 5 µl of each of three solutions in *dichloromethane* containing (1) 4.0% w/v of the substance being examined, (2) 0.020% w/v of the substance being examined and (3) 0.020% w/v of *theophylline*. After removal of the plate, dry it in a current of cold air and examine under *ultraviolet light (254 nm)*. Any spot corresponding to theophylline in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). Spray the plate with *potassium iodobismuthate solution*, allow it to dry in air and spray with *hydrogen peroxide solution (10 vol)*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (2). Disregard any spot extending from the line of application to an Rf value of about 0.1.

Loss on drying When dried to constant weight over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa, loses not more than 0.5% of its weight. Use 1 g.

Sulphated ash Not more than 0.2%, Appendix IX A, Method II. Use 1 g.

Assay ***For diphenhydramine*** Dissolve 0.2 g in 60 ml of *anhydrous acetic acid* and carry out Method I for *non-aqueous titration*, Appendix VIII A, determining the end point potentiometrically. Each ml of 0.1M *perchloric acid VS* is equivalent to 25.54 mg of $C_{17}H_{21}NO$.

For 8-chlorotheophylline To 0.8 g add 50 ml of *water*, 3 ml of 6M *ammonia* and 0.6 g of *ammonium nitrate* and heat on a water bath for 5 minutes. Add 25 ml of 0.1M *silver nitrate VS* and continue heating on a water bath for 15 minutes with frequent swirling. Cool, add 25 ml of 2M *nitric acid*, dilute to 250 ml with *water*, filter and discard the first 25 ml of the filtrate. Titrate 100 ml of the filtrate with 0.1M *ammonium thiocyanate VS* using 5 ml of *ammonium iron(III) sulphate solution R2* as indicator until the colour changes to yellowish brown. Each ml of 0.1M *silver nitrate VS* is equivalent to 21.46 mg of $C_7H_7ClN_4O_2$.

Preparations
Dimenhydrinate Injection
Dimenhydrinate Tablets

Action and use Antiemetic.

by subcutaneous injection in addition to ...idazole 50 mg per kg daily or niridazole ... daily, both drugs being given for 10 ... Scragg and S. J. Powell, *Archs Dis.* *45*, 193, per R. Knight *et al.*, *Gut*, 1973, ...

... Twenty elderly patients with herpes ... experience postherpetic neuralgia, and in ...pletely disappeared after treatment with ...e 60 mg given intramuscularly every 24 ... to 9 doses. In the other 6 patients pain ... disappeared in 2 to 4 weeks. No patients ...cles at the end of the first week of treat... similar patients treated with triamcinolone ...experienced postherpetic neuralgia and pain ...more than 6 months in 4.— E. Hernan...*is*, 1980, *25*, 424.

... hydrochloride is marketed in certain ...r the proprietary names Dametine (*E.* ...Dehydroemetine Roche.

... **Acetarsol.** Diethylamine Acetarsone; ...arsine. The dihydrate of the diethylamine ...imido-4-hydroxyphenylarsonic acid.
...$C_4H_{11}N,2H_2O$ = 384.3.
...*3-8 (anhydrous).*
...*las.* In *Belg.*

...ystals or a white crystalline odourless ... slightly bitter taste. **Soluble** 1 in 3.5 of ... of boiling water, and 1 in 7 of alcohol; ...soluble in chloroform and ether.

...acetarsol was formerly used in the treat...bite fever, relapsing fever, tropical eosinoph... dermatoses.

...acetarsol was formerly marketed in certain ...r the proprietary name Acetylarsan (*May* ...

... **Sodium.** Diphetarsone; RP 4763. Diso...ethylenebis(4-aminophenylarsonate) decahyd...
...$Na_2O_6,10H_2O$ = 684.3.
...*19-19-8 (difetarsone); 515-76-4 (sodium salt,* ...

...odium has been used in the treatment of ...moebiasis, usually in conjunction with a ...rum antibiotic, in divided doses of up to 2 g ...0 days repeated after 5 to 6 weeks, if neces... also been used in the treatment of whip... threadworm infection. See also Difetarsone... (below).

...o the treatment of whipworm infection: D. ...*et al.*, *Br. med. J.*, 1972, *4*, 73; N. M. ...(letter), *Trans. R. Soc. trop. Med. Hyg.*, 1972, ...R. O'Holohan and J. Hugoe-Matthews, *S.E. ...op. med. publ. Hlth*, 1972, *3*, 576, per *Trop.* ...1973, *70*, 669; C. J. Rubidge *et al.*, *S. Afr.* ...73, *47*, 991, per *Trop. Dis. Bull.*, 1973, *70*, ...Leary *et al.*, *Archs Dis. Childh.*, 1974, *49*, ...

...Names
...*Specia, Fr.).*

... **-Spiramycin.** Diphetarsone-Spiramycin; ...e spiramycin salt of difetarsone, containing ...piramycin base and 33.8% of difetarsone.

...spiramycin has been used similarly to dife... in the treatment of intestinal amoebiasis ...dworm and whipworm infestation.

...Names
...Difétarsone *(Specia, Fr.).*

4777-m

Di-iodohydroxyquinoline *(B.P. 1973).*

Iodoquinol *(U.S.P.)*; Diiodohydroxyquin; Diiodohydroxyquinolinum; Diodoxyquinoléine. 5,7-Di-iodoquinolin-8-ol.

$C_9H_5I_2NO$ = 397.0.

CAS — 83-73-8.

Pharmacopoeias. In *Chin.*, *Fr.*, *Ind.*, *Int.*, *It.*, and *U.S.*

A light yellowish to tan-coloured, tasteless, microcrystalline powder, not readily wetted in water, odourless or with a slight odour.

Practically **insoluble** in water; sparingly soluble in alcohol, acetone, and ether. **Protect** from light.

Adverse Effects. As for Clioquinol, p.975.

Effects occasionally occurring include abdominal discomfort, diarrhoea, skin rash, acne, headache, pruritus ani, and furunculosis. Slight enlargement of the thyroid gland often occurs during treatment.

Neurological disorders. Reports of visual disturbances in children given di-iodohydroxyquinoline.— J. E. Etheridge and G. T. Stewart (letter), *Lancet*, 1966, *1*, 261; F. E. Pittman and M. Westphal (letter), *Lancet*, 1973, *2*, 566; M. M. Behrens (letter), *J. Am. med. Ass.*, 1974, *228*, 693.

Precautions. As for Clioquinol, p.975.

Control of acrodermatitis enteropathica by di-iodohydroxyquinoline was lost in a patient when she started taking an oral contraceptive.— M. J. Jackson, *J. clin. Path.*, 1977, *30*, 284.

Absorption and Fate. Di-iodohydroxyquinoline is partly and irregularly absorbed from the small intestine.

Following a 300-mg dose of di-iodohydroxyquinoline, 6 healthy men excreted a mean of 4.6% of the dose in the urine as glucuronide during the following 10 hours.— L. Berggren and O. Hansson, *Clin. Pharmac. Ther.*, 1968, *9*, 67.

Uses. Di-iodohydroxyquinoline acts principally in the bowel lumen and is used alone or with metronidazole in the treatment of intestinal amoebiasis, chiefly for cyst-passers. It has been used to supplement emetine or with chloroquine and tetracycline in amoebic dysentery. It has also been used in balantidiasis and giardiasis and has been used locally against *Trichomonas vaginalis*. Di-iodohydroxyquinoline has been used in the treatment of acrodermatitis enteropathica; it is reported to act by altering zinc absorption.

The usual dosage in the treatment of amoebiasis is 600 mg thrice daily for 20 days; for children the usual dose is 10 mg per kg body-weight thrice daily. It can be employed in ambulatory patients and asymptomatic carriers.

Most of 55 patients with ocreiform atrophy and superimposed dermatitis of the anterior surface of the lower leg responded well to an ointment containing di-iodohydroxyquinoline 3% and salicylic acid 2% in Emulsifying Ointment.— A. R. H. B. Verhagen and J. W. Koten, *Br. J. Derm.*, 1968, *80*, 682.

Di-iodohydroxyquinoline should not be used for the treatment of non-specific diarrhoea or other self-limiting conditions.— *Med. Lett.*, 1974, *16*, 71.

Acrodermatitis. A 5-month-old girl with acrodermatitis enteropathica obtained remission from diarrhoea and dermatitis when treated with di-iodohydroxyquinoline 200 mg thrice daily. She relapsed and was then given a diet of fresh whole human milk; treatment with di-iodohydroxyquinoline was continued in the same dosage. On this regimen a complete remission was obtained enabling the child to be weaned to a normal diet and treatment with di-iodohydroxyquinoline to be discontinued.— R. R. Schulze and R. K. Winkelmann, *Mayo Clin. Proc.*, 1966, *41*, 334.

In acrodermatitis enteropathica di-iodohydroxyquinoline acted by increasing the gastro-intestinal absorption as well as the retention of zinc.— M. J. Jackson, *J. clin. Path.*, 1977, *30*, 284. See also P. J. Aggett *et al.*, *Archs Dis. Childh.*, 1978, *53*, 691.

Aspergillosis. Of 13 patients with clinical pulmonary aspergillosis all had specific precipitins in their sera and most had *Aspergillus fumigatus* in their sputum. After treatment for 20 days with di-iodohydroxyquinoline 1.5 to 1.8 g daily precipitin tests became negative in 12 and the sputum was cleared in all those previously affected. Some patients experienced clinical benefit.— K. Horsfield *et al.*, *Thorax*, 1977, *32*, 250, per *Abstr. Hyg.*, 1977, *52*, 1131.

Preparations

Di-iodohydroxyquinoline Pessaries *(B.P.C. 1973).* Each pessary contains di-iodohydroxyquinoline 100 mg, boric acid 65 mg, phosphoric acid 17 mg, lactose 180 mg, and anhydrous dextrose 300 mg; prepared by moist granulation and compression. They should be moistened with water before insertion into the vagina. Protect from light. *A.P.F.* has a similar formula.

Di-iodohydroxyquinoline Tablets *(B.P. 1973).* Di-iodohydroxyquin. Tab. Tablets containing di-iodohydroxyquinoline. Protect from light.

Iodoquinol Tablets *(U.S.P.).* Tablets containing di-iodohydroxyquinoline.

Proprietary Preparations

Diodoquin *(Searle, UK).* Di-iodohydroxyquinoline, available as tablets of 650 mg. (Also available as Diodoquin in many other countries).

Embequin *(May & Baker, UK).* (Available only in certain countries.) Di-iodohydroxyquinoline, available as tablets of 300 mg.

Other Proprietary Names

Dioxiquin *(Spain)*; Direxiode *(Austral., Belg., Fr., Switz.)*; Drioquilen *(Arg.)*; Floraquin *(Arg., Austral., Belg.)*; Moebiquin *(USA)*; Searlequin *(Arg.)*; Yodoxin *(USA)*.

A preparation containing di-iodohydroxyquinoline was formerly marketed in Great Britain under the proprietary name Floraquin (*Searle Pharmaceuticals*).

4778-b

Diloxanide *(B.P.C. 1963).*

Diloxan; RD 3803. 2,2-Dichloro-4′-hydroxy-*N*-methylacetanilide.

$C_9H_9Cl_2NO_2$ = 234.1.

CAS — 579-38-4.

A white or almost white, odourless, tasteless, crystalline powder. Slightly **soluble** in water; soluble 1 in 8 of alcohol, 1 in 35 of chloroform, and 1 in 66 of ether. **Protect** from light.

The actions and uses of diloxanide are described under Diloxanide Furoate (below). It has been given in doses of 1.5 g daily in divided doses.

Diloxanide was formerly marketed in certain countries under the proprietary name Entamide (*Boots*).

4779-v

Diloxanide Furoate *(B.P.).*

4-(*N*-Methyl-2,2-dichloroacetamido)phenyl 2-furoate.

$C_{14}H_{11}Cl_2NO_4$ = 328.2.

CAS — 3736-81-0.

Pharmacopoeias. In *Br.*

A white or almost white, odourless, tasteless, crystalline powder. M.p. 114° to 116°.

Very slightly **soluble** in water; soluble 1 in 100 of alcohol, 1 in 2.5 of chloroform, and 1 in 130 of ether. **Protect** from light.

Adverse Effects. Flatulence, vomiting, pruritus, and urticaria may occasionally occur. Transient albuminuria has been reported.

Absorption and Fate. Diloxanide is readily absorbed from the gastro-intestinal tract and excreted in the faeces and urine. Diloxanide furoate is hydrolysed before absorption.

Uses. Diloxanide acts principally in the bowel lumen and is used in the treatment of intestinal amoebiasis. It is less effective in amoebic dysentery than in asymptomatic infection, but the furoate gives higher intestinal concentrations and is possibly more effective than metronidazole in the treatment of cyst-passers.

Diloxanide furoate is used in conjunction with chloroquine and tetracycline in amoebic dysentery and is used in the treatment of hepatic amoebiasis in conjunction with chloroquine and

dehydroemetine or emetine.

Diloxanide furoate is administered in a dosage of 500 mg thrice daily for 10 days. The dosage for children is 20 mg per kg body-weight daily, in divided doses, for 10 days. The course of treatment may be repeated if necessary.

Diloxanide furoate is also used concomitantly with metronidazole.

Amoebiasis. Diloxanide furoate 375 mg, tetracycline hydrochloride 250 mg, and chloroquine phosphate 100 mg, 4 times daily for 5 days, were given in capsules to 50 of 100 patients with dysentery due to *Entamoeba histolytica* and sometimes other parasites also. The other 50 received the same regimen without chloroquine. Children younger than 10 years received half this adult dose. The overall cure-rate for *E. histolytica* was 83%, and the efficacy of the preparations was not significantly different. Other protozoa and helminths were apparently not affected.— D. Botero, *Trans. R. Soc. trop. Med. Hyg.*, 1967, *61*, 769, per *Abstr. Wld Med.*, 1968, *42*, 497.

Diloxanide furoate 375 mg, tetracycline hydrochloride 250 mg, and chloroquine phosphate 100 mg, given 4 times daily for 5 days to 50 Costa Rican schoolboys, eliminated multiple intestinal protozoal infections within 2 days of completing the course. The recurrence-rate of *Giardia intestinalis* was 25% within 30 days, but *Entamoeba histolytica* did not recur for 90 days.— M. M. Schapiro, *Am. J. trop. Med. Hyg.*, 1967, *16*, 704, per *Trop. Dis. Bull.*, 1968, *65*, 766. A similar report.— E. Nnochiri, *J. trop. Med. Hyg.*, 1967, *70*, 224, per *Trop. Dis. Bull.*, 1968, *65*, 129.

Diloxanide furoate administered in a dose of 500 mg thrice daily for 10 days was effective in the treatment of 12 patients who were asymptomatic cyst carriers and 52 of 65 patients with non-dysenteric symptomatic intestinal amoebiasis. Flatulence was the only significant side-effect.— M. S. Wolfe, *J. Am. med Ass.*, 1973, *224*, 1601.

Diloxanide furoate was considered to be more effective than metronidazole in the treatment of non-dysenteric intestinal amoebiasis, and to be the drug of choice for this form of the disease.— R. Knight *et al.*, *Gut*, 1973, *14*, 145.

Diloxanide furoate 500 mg given with metronidazole 400 mg thrice daily for 5 days cleared amoebic cysts from the intestine in 59 of 60 patients treated and was considered to have cured liver abscesses in 58 of them. No relapses were noted during 3 months following treatment.— S. I. Powell *et al.*, *Ann. trop. Med. Parasit.*, 1973, *67*, 367, per *Trop. Dis. Bull.*, 1974, *71*, 44.

The standard regimen for the treatment of amoebiasis in American Indians in Saskatchewan was metronidazole 500 mg and diloxanide furoate 500 mg twice daily for 5 days.— R. D. P. Eaton *et al.*, *Can. J. publ. Hlth*, 1973, *64. Suppl.*, 47, per *Trop. Dis. Bull.*, 1974, *71*, 360.

Of 38 Peace Corps workers with amoebiasis in Ethiopia 36 were considered free of infection 1 to 2 months after treatment with metronidazole 750 mg thrice daily for 10 days followed by diloxanide furoate 500 mg thrice daily for 10 days.— J. L. Ey, *Ethiop. med. J.*, 1977, *15*, 101, per *Trop. Dis. Bull.*, 1979, *76*, 80.

A report of the successful treatment of a patient with *Entamoeba polecki* infection using metronidazole and diloxanide furoate.— J. S. Salaki *et al.*, *Am. J. trop. Med. Hyg.*, 1979, *28*, 190, per *Trop. Dis. Bull.*, 1980, *77*, 51.

Preparations

Diloxanide Furoate Tablets *(B.P.)*. Tablets containing diloxanide furoate. Protect from light.

Furamide *(Boots, UK)*. Diloxanide furoate, available as tablets of 500 mg. (Also available as Furamide in *Austral*).

4780-r

Diminazene Aceturate *(B. Vet. C. 1965)*. 1,3-Bis(4-amidinophenyl)triazene bis(*N*-acetylglycinate) tetrahydrate.

$C_{22}H_{29}N_9O_6,4H_2O = 587.6$.

CAS — 536-71-0 (diminazene); 908-54-3 (aceturate, anhydrous).

A yellow odourless powder. **Soluble** 1 in 14 of water; slightly soluble in alcohol; very slightly soluble in chloroform and ether.

Uses. Diminazene aceturate has trypanocidal, babesicidal, and bactericidal properties and is used in veterinary medicine in the treatment of trypanosomiasis and babesiasis. It has also been tried in human infections.

Babesiasis. The routine clinical use of pentamidine or diminazene aceturate in infections due to *Babesia microti* was not recommended except in patients without spleens, since normally the infection was self-limiting.— L. H. Miller *et al.*, *Ann. intern. Med.*, 1978, *88*, 200.

A patient infected with *Babesia microti* who had failed to respond to chloroquine had a rapid clinical and parasitologic response after administration of diminazene. However the patient developed Guillain-Barré syndrome after treatment and it was suggested that pentamidine might be preferable to diminazene in severe cases of human babesiasis.— T. K. Ruebush and A. Spielman, *Ann. intern. Med.*, 1978, *88*, 263.

Trypanosomiasis. Reference to use in human trypanosomiasis.— M. P. Hutchinson and H. J. C. Watson, *Trans. R. Soc. trop. Med. Hyg.*, 1962, *56*, 227; S. E. Temu, *Trans. R. Soc. trop. Med. Hyg.*, 1975, *69*, 277; East African Trypanosomiasis Research Organisation, *Trans. R. Soc. trop. Med. Hyg.*, 1975, *69*, 278.

Proprietary Names

Berenil *(veterinary) (Hoechst, UK)*; Ganaseg.

4781-f

Emetine and Bismuth Iodide *(B.P. 1973)*. Emet. Bism. Iod.; EBI.

CAS — 8001-15-8.

A complex iodide of emetine and bismuth containing 25 to 30% of emetine and 18 to 22.5% of Bi. It is a reddish-orange odourless powder with a bitter acrid taste.

Practically **insoluble** in water and alcohol; soluble in acetone and, with decomposition, in concentrated acids and in alkaline solutions; practically insoluble in but slightly decomposed by dilute acids. **Store** in airtight containers. Protect from light.

Adverse Effects and Precautions. As for Emetine Hydrochloride (below).

When given by mouth emetine and bismuth iodide may cause nausea, vomiting, and diarrhoea.

Absorption and Fate. When given by mouth, emetine and bismuth iodide undergoes little decomposition until it reaches the small intestine, where emetine is liberated and exerts a local and systemic effect.

Uses. Emetine and bismuth iodide has actions similar to those of emetine hydrochloride and has been used in the treatment of asymptomatic intestinal amoebiasis. When given by mouth it is only slightly decomposed before reaching the small intestine where the bulk of the emetine is then released to give a high concentration in the intestine. It has been used with tetracycline and a luminal amoebicide such as diloxanide furoate in the treatment of severe amoebic dysentery with much tissue invasion.

The frequency with which it gives rise to unpleasant side-effects makes it unsuitable for routine therapy; patients should be confined to bed.

Emetine and bismuth iodide is usually administered in enteric-coated tablets or capsules but such preparations must disintegrate very readily in the intestine or they are valueless; when in capsules, the drug should not be suspended in an oily basis. The usual dose was 200 mg daily for 12 consecutive days if tolerated by the patient.

Preparations

Emetine and Bismuth Iodide Tablets *(B.P. 1973)*. Emet. Bism. Iod. Tab. Tablets containing emetine and bismuth iodide. They are enteric-and sugar-coated. Store at a temperature not exceeding 25° in airtight containers.

4782-d

Emetine Hydrochloride *(B.P., U.S.P.)*.

Emet. Hydrochlor.; Emetini Hydrochloridum; Emetini Chloridum; Emetine Dihydrochloride; Ipecine Hydrochloride; Methylcephaëline Hydrochloride; Cloridrato de Emetina. 6′,7′,10,11-Tetramethoxyemetan dihydrochloride heptahydrate; (2*S*,3*R*,11b*S*)-3-Ethyl-1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-2-[(1*R*)-1,2,3,4-tetrahydro-6,7-dimethoxy-1-isoquinolylmethyl]-2*H*-benzo[*a*]-quinolizine dihydrochloride heptahydrate.

$C_{29}H_{40}N_2O_4,2HCl,7H_2O = 679.7$.

CAS — 483-18-1 (emetine); 316-42-7 (hydrochloride, anhydrous); 7083-71-8 (hydrate).

Pharmacopoeias. In *Arg.*, *Aust.*, *Ger.*, *Ind.*, *Int.*, *It.*, *Jug.*, *Mex.*, *Rus.*, *Span.*, *Swiss*, *Turk.*, and *U.S.*, variable proportion of water of cr...

A white or very slightly yellow... line powder with a bitter taste... yellow on exposure to light... after drying.

Soluble 1 in 8 of water, 1 in ... and 1 in 4 of chloroform; prac... ether. A solution in water ... 2% solution in water has a pH ... tions are **sterilised** by maintain... for 30 minutes with a bactericide. **Store** in airtight containers. Pro...

The stability of emetine hydroch... Schuyt *et al.*, *Pharm. Weekbl. Ned. idem*, 1979, *114*, 186.

Adverse Effects. Emetine caus... and there may be associated ... there may be necrosis and fa... After injection nausea, vomiting... are common; there may be dizz... muscle weakness, urticaria or ... and, more rarely, mild sensory ... Cardiovascular effects are consid... include precordial pain, dyspno... and hypotension. Changes in ... cularly flattening or inversion of ... prolongation of the Q-T interval ... patients. Large doses or prolonged ... may cause lesions of the heart ... tract, kidneys, liver, and skeletal ... acute degenerative myocarditis ... may give rise to sudden cardi... death. In some patients cardiotox... appeared after the completion of ... therapeutic doses.

Four patients given emetine for ... muscular weakness or peripheral ... doses ranging from 180 to 720 mg ... D. S. Yeoh, *Singapore med. J.*, 1966... *Dis. Bull.*, 1968, *65*, 32.

Precautions. Emetine is cont... cardiac or renal disease. It ... avoided during pregnancy and ... given to children, except ... dysentery unresponsive to other ... be used with great caution in ... patients. ECG monitoring is ... treatment.

Absorption and Fate. After ... hydrochloride is concentrated in ... ciable concentration occurs also ... and spleen. Very little of an ... secreted into the intestinal lumen ... urs mainly in the urine, and is ... concentrations may persist in uri... after treatment has been discont... tion may occur.

Uses. Emetine, an alkaloid of ipe... amoebicide acting principally in ... and in the liver. It is given by ... intramuscular injection. In intest... the symptoms are rapidly cleared ... course of emetine injections and ... and cysts disappear, but mor... patients later show cysts in th... hence become carriers. Further ... emetine hydrochloride in these ca... value.

In severe amoebic dysentery it may ... tetracycline and an amoebicide act... intestinal lumen such as diloxanide ... In hepatic amoebiasis emetine may ... chloroquine and an amoebicide act... intestinal lumen, but treatment ... ronidazole is generally preferred. ... Doses of emetine hydrochloride ... larger than 60 mg daily and cou... be longer than 10 days or repeated ...

Di-iodohydroxyquinoline has also been given in the treatment of balantidiasis as an alternative to tetracycline (see p.610).

Di-iodohydroxyquinoline was formerly used in the treatment of acrodermatitis enteropathica; it is reported to act by enhancing zinc absorption and has now been superseded by oral zinc therapy (see p.1395).

Di-iodohydroxyquinoline has antibacterial and antifungal activity and has been used topically in various skin conditions, usually together with a corticosteroid (but see under Adverse Effects p.616). It also has some antitrichomonal activity.

Amoebic Infections. As discussed on p.609 di-iodohydroxyquinoline is one of the drugs used in the treatment of intestinal amoebiasis caused by *Entamoeba histolytica* and *Dientamoeba fragilis*. References to this use are given below.

1. Anonymous. Drugs for parasitic infections. *Med Lett Drugs Ther* 1993; **35**: 111–22.

Preparations

Names of preparations are listed below; details are given in Part 3.

Official Preparations
BP 93: Iodoquinol Tablets.

Proprietary Preparations
Canad.: Diodoquin; Yodoxin†; *Fr.:* Direxiode†; *S.Afr.:* Floraquin; *USA:* Sebaquin†; Yodoxin.

Multi-ingredient preparations. *Austral.:* Floraquin; *Canad.:* Entoquinol†; *S.Afr.:* Vagarsol; Viocort; Viodor; *Spain:* Floraquin; *USA:* Wynone.

Diloxanide Furoate (4779-v)

Diloxanide Furoate *(BANM, rINNM)*.

4-(*N*-Methyl-2,2-dichloroacetamido)phenyl 2-furoate.

$C_{14}H_{11}Cl_2NO_4 = 328.2$.

CAS — 579-38-4 (diloxanide); 3736-81-0 (diloxanide furoate).

Pharmacopoeias. In *Br.* and *Int.*

A white or almost white, odourless, crystalline powder. Very slightly soluble in water; slightly soluble in alcohol and in ether; freely soluble in chloroform. **Protect** from light.

Adverse Effects

Flatulence is the most common adverse effect during treatment with diloxanide furoate. Vomiting, pruritus, and urticaria may occasionally occur.

Pharmacokinetics

Diloxanide furoate is hydrolysed before absorption from the gastro-intestinal tract. The resulting diloxanide is readily absorbed and excreted mainly in the urine; less than 10% of a dose appears in the faeces.

Uses and Administration

Diloxanide furoate, a dichloroacetamide derivative, is a luminal amoebicide acting principally in the bowel lumen and is used in the treatment of intestinal amoebiasis. It is given alone in the treatment of asymptomatic cyst-passers and in conjunction with an amoebicide that acts in the tissues, such as metronidazole, in patients with invasive amoebiasis. Diloxanide furoate has also been used with metronidazole in the treatment of *Entamoeba polecki* infection.

For further discussion of the management of amoebic infections, see p.609.

Diloxanide furoate is administered by mouth in a dosage of 500 mg three times daily for 10 days; children may be given 20 mg per kg body-weight daily, in divided doses, for 10 days. The course of treatment may be repeated if necessary.

Preparations

Names of preparations are listed below; details are given in Part 3.

Official Preparations
BP 1993: Diloxanide Tablets.

Proprietary Preparations
Austral.: Furamide†; *Switz.:* Furamid; *UK:* Furamide.

Multi-ingredient preparations. *UK:* Entamizole.

Dimetridazole (12662-z)

Dimetridazole *(BAN, pINN)*.

1,2-Dimethyl-5-nitroimidazole.

$C_5H_7N_3O_2 = 141.1$.

CAS — 551-92-8.

Pharmacopoeias. In *BP(Vet)*.

Cz. includes Dimetridazole for veterinary use only. *Fr.* includes Dimetridazole and Dimetridazole Mesylate for veterinary use only.

An almost white to brownish-yellow, odourless or almost odourless powder which darkens on exposure to light. Slightly **soluble** in water; sparingly soluble in alcohol; freely soluble in chloroform; slightly soluble in ether. **Protect** from light.

Dimetridazole is a 5-nitroimidazole derivative similar to metronidazole. It is used in veterinary practice for the prevention and treatment of blackhead (histomoniasis) in turkeys and other poultry and of swine dysentery, and for the prevention of hexamitiasis and trichomoniasis in game birds.

Diminazene Aceturate (4780-r)

Diminazene Aceturate *(BANM, rINNM)*.

1,3-Bis(4-amidinophenyl)triazene bis(*N*-acetylglycinate).

$C_{22}H_{29}N_9O_6 = 515.5$.

CAS — 536-71-0 (diminazene); 908-54-3 (diminazene aceturate).

NOTE. Diminazene aceturate is often referred to by its veterinary proprietary name Berenil.

Diminazene aceturate, an aromatic diamidine derivative related to pentamidine, is an antiprotozoal agent which has been used in veterinary medicine in the treatment of trypanosomiasis and babesiosis. It has also been tried in human infections.

References.

1. Ruebush TK, Spielman A. Human babesiosis in the United States. *Ann Intern Med* 1978; **88**: 263.
2. Abaru DE, *et al.* Retrospective long-term study of effects of Berenil by follow-up of patients treated since 1965. *Trop Med Parasitol* 1984; **35**: 148–50.

Residues in the diet. An expert committee of the FAO/WHO[1] set a maximum acceptable daily intake of diminazene at 100 µg per kg body-weight. Recommended maximum residue limits in food resulting from veterinary use were established for cattle at 500 µg per kg for muscle, 12 000 µg per kg for liver, 6000 µg per kg for kidney, and 150 µg per litre for milk.

1. FAO/WHO. Evaluation of certain veterinary drug residues in food: forty-second report of the joint FAO/WHO expert committee on food additives. *WHO Tech Rep Ser 851* 1995.

Dinitolmide (12665-a)

Dinitolmide *(BAN, rINN)*.

Dinitrotoluamide; Methyldinitrobenzamide. 3,5-Dinitro-*o*-toluamide.

$C_8H_7N_3O_5 = 225.2$.

CAS — 148-01-6.

Pharmacopoeias. In *BP(Vet)*.

A cream-coloured to light tan-coloured odourless powder. Practically **insoluble** in water; soluble in acetone; slightly soluble in alcohol, in chloroform, and in ether.

Dinitolmide is an antiprotozoal agent used in veterinary practice for the prevention of coccidiosis in poultry.

Eflornithine Hydrochloride (16604-j)

Eflornithine Hydrochloride *(BANM, USAN, rINNM)*.

DFMO; α-Difluoromethylornithine Hydrochloride; MDL-71782; MDL-71782A; RMI-71782. 2-(Difluoromethyl)-DL-ornithine monohydrochloride monohydrate.

$C_6H_{12}F_2N_2O_2.HCl.H_2O = 236.6$.

CAS — 67037-37-0 (eflornithine); 96020-91-6 (eflornithine hydrochloride).

Adverse Effects and Precautions

Reported adverse effects with eflornithine include myelosuppression producing anaemia, leucopenia, and thrombocytopenia. Some patients have experienced hearing loss and alopecia. Gastro-intestinal disturbances, especially diarrhoea, can be a problem with oral administration. Seizures have occurred in about 8% of patients given eflornithine but they may have been related to the disease rather than treatment.

Effects on hearing. A study in 58 patients[1] receiving eflornithine alone or in combination with interferon alfa for the treatment of metastatic melanoma demonstrated that hearing loss at multiple frequencies was related to the cumulative dose of eflornithine and was worse in patients with pre-existing hearing deficit.

1. Croghan MK, *et al.* Dose-related α-difluoromethylornithine ototoxicity. *Am J Clin Oncol* 1991; **14**: 331–5.

Effects on the heart. Fatal cardiac arrest occurred in an AIDS patient with *Pneumocystis carinii* pneumonia during the intravenous infusion of eflornithine 100 mg per kg body-weight over 1 hour.[1] Sudden death after infusion of eflornithine had occurred in several other critically ill patients with AIDS.

1. Barbarash RA, *et al.* Alpha-difluoromethylornithine infusion and cardiac arrest. *Ann Intern Med* 1986; **105**: 141–2.

Pharmacokinetics

Eflornithine hydrochloride is absorbed from the gastro-intestinal tract. Following intravenous administration approximately 80% is excreted unchanged in the urine in 24 hours. The terminal elimination half-life is approximately 3 hours. It is distributed to the CSF.

References.

1. Haegele KD, *et al.* Kinetics of α-difluoromethylornithine: an irreversible inhibitor of ornithine decarboxylase. *Clin Pharmacol Ther* 1981; **30**: 210–17.
2. Milord F, *et al.* Eflornithine concentrations in serum and cerebrospinal fluid of 63 patients treated for Trypanosoma brucei gambiense sleeping sickness. *Trans R Soc Trop Med Hyg* 1993; **87**: 473–7.

Uses and Administration

Eflornithine hydrochloride is an antiprotozoal agent which acts as an irreversible inhibitor of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis; trypanosomes are more susceptible to the effects of eflornithine than humans probably because of their slower turnover of this enzyme.

Eflornithine is used in African trypanosomiasis (p.613) mainly due to *Trypanosoma brucei gambiense* and is effective in the early and more importantly in the late stage of the disease when there is central involvement. Eflornithine also has activity against *Pneumocystis carinii* (see p.396) and there are several reports of it being effective in patients whose pneumonia due to this organism failed to respond to standard treatment such as co-trimoxazole or pentamidine.

It is administered intravenously or by mouth, though diarrhoea can be troublesome with the latter route. The usual dose is 100 mg per kg body-weight every 6 hours by intravenous infusion for 14 days. Some clinicians then give 300 mg per kg per day by mouth for a further 3 to 4 weeks. Dosage should be reduced in patients with impaired renal function.

Eflornithine has antineoplastic activity, and preliminary human studies have shown some encouraging responses.

Cryptosporidiosis. Eflornithine has been tried in the treatment of cryptosporidiosis in AIDS patients.[1] Other agents used in the treatment of cryptosporidiosis are discussed on p.610.

1. Rolston KVI, *et al.* Intestinal cryptosporidiosis treated with eflornithine: a prospective study among patients with AIDS. *J Acquir Immune Defic Syndr* 1989; **2**: 426–30.

Pneumocystis carinii pneumonia. The treatment of *Pneumocystis carinii* pneumonia is described on p.396 where reference is made to eflornithine being studied as one of the alternative agents to co-trimoxazole and pentamidine.

References.

Database: Medline <1966 to present>

Set	Search	Results
1	diloxanide furoate.tw.	30
2	stability.tw.	54760
3	1 and 2	0
4	from 1 keep 2,4-5,7,12,15,17,19-21,28	11

<1>
Unique Identifier
97321428
Authors
Qureshi H. Ali A. Baqai R. Ahmed W.
Title
Efficacy of a combined diloxanide furoate-metronidazole preparation in the treatment of amoebiasis and giardiasis.
Source
Journal of International Medical Research. 25(3):167-70, 1997 May-Jun.
Abstract
A combined formulation of diloxanide furoate and metronidazole was used to treat amoebiasis and giardiasis (cysts and vegetative forms) in 54 patients. Of these 34 patients had amoebiasis, 19 had giardiasis and one had mixed infection. Each patient took one tablet (containing 500 mg diloxanide furoate and 400 mg metronidazole), three times daily for 5 days, and the response to therapy was checked by clinical examination and by examination of fresh stools on days 3, 5 and 10. Abdominal pain was completely relieved in 91% and 84% of patients with amoebiasis and giardiasis, respectively, while parasitic clearance was 100% in both groups. Tolerance to the drug was adequate.

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Authors
Bhopale KK. Pradhan KS. Masani KB. Kaul CL.
Title
Additive effect of diloxanide furoate and metronidazole (Entamizole) in experimental mouse caecal amoebiasis.
Source
Indian Journal of Experimental Biology. 33(1):73-4, 1995 Jan.

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Unique Identifier
96319050
Authors
Sengupta M. Sengupta O.
Title
Correlation of biological activity (therapeutic and toxic) with solvochromic properties of metronidazole, emetine hydrochloride and diloxanide furoate.
Source
Indian Journal of Biochemistry & Biophysics. 32(5):302-7, 1995 Oct.
Abstract
Goat blood, when incubated for different periods with diloxanide furoate, metronidazole and emetine hydrochloride, underwent changes in fatty acid constituents and their peroxidation products measured as malonaldehyde. These findings, together with the changes noted in the drug-lipid partition coefficient, are discussed in an attempt to correlate the lipid constitution and biological activity of the drugs.

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Authors
Pehrson P. Bengtsson E.
Title
Treatment of non-invasive amoebiasis. A comparison between tinidazole alone and in combination with diloxanide furoate.
Source
Transactions of the Royal Society of Tropical Medicine & Hygiene. 77(6):845-6, 1983.
Abstract
Tinidazole (40 mg/kg body-weight in one daily dose for five days) and tinidazole (same dose) plus diloxanide furoate (20 mg/kg body-weight divided into three daily doses for 10 days) were compared as treatments for amoebiasis. The parasitic cure rates were 44 and 91% respectively. We cannot, therefore, recommend tinidazole alone in this dosage as a treatment for non-invasive amoebiasis.

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Authors
Salaki JS. Shirey JL. Strickland GT.
Title
Successful treatment of symptomatic Entamoeba polecki infection.
Source
American Journal of Tropical Medicine & Hygiene. 28(2):190-3, 1979 Mar.
Abstract
The second symptomatic case of Entamoeba polecki infection, the first to respond to therapy, is reported. The patient experienced intermittent episodes of abdominal cramps, diarrhea, nausea, and malaise associated with large numbers of E. polecki cysts in the stool. Following treatment with diloxanide furoate and metronidazole, all symptoms cleared and the parasite was no longer present in the stool.

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Unique Identifier
73184480
Authors
Wolfe MS.
Title
Nondysenteric intestinal amebiasis. Treatment with diloxanide furoate.
Source
JAMA. 224(12):1601-4, 1973 Jun 18.

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Unique Identifier
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Authors
Botero D.
Title
Treatment of intestinal amoebiasis with diloxanide furoate, tetracycline and chloroquine.
Source
Transactions of the Royal Society of Tropical Medicine & Hygiene. 61(6):769-73, 1967.

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Authors
Huggins D.

Title
[Treatment of amebiasis. Results obtained with diloxanide furoate]. [Portuguese]
Source
Revista do Instituto de Medicina Tropical de Sao Paulo. 7(2):110-1, 1965 Mar-Apr.

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Authors
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[Treatment of amebiasis. (Results obtained with diloxanide furoate)]. [Portuguese]
Source
Hospital. 67(5):1107-10, 1965 May.

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95048473
Authors
Burchard GD.
Title
[Therapy for malaria and amoebiasis]. [Review] [12 refs] [German]
Source
Immunitat und Infektion. 22(2):45-7, 1994 Apr.
Abstract
Treatment of malaria depends on the infecting Plasmodium species. In Plasmodium falciparum malaria the treatment also depends on whether chloroquine resistances occur and whether the course is uncomplicated or complicated. Uncomplicated cases are cared for with chloroquine and with mefloquine or halofantrine when the patient comes from areas with chloroquine resistances. Patients with complicated Plasmodium falciparum malaria must get chinine and doxycycline. A careful fluid balance is extremely important in order to prevent noncardiac pulmonary edemas. Luminal infections with pathogenic Entamoeba histolytica are treated with diloxanide furoate, luminal infections with non-pathogenic Entamoeba histolytica (= E. dispar) do not have to be treated. If differentiation is not possible, all asymptomatic cyst passers must get treatment. Patients with invasive amebiasis (amebic colitis and amebic liver abscess) have to be treated with metronidazole, followed by diloxanide furoate. [References: 12]

<11>
Unique Identifier
89279444
Authors
Di Perri G. Strosselli M. Rondanelli EG.
Title
Therapy of entamebiasis.
Source
Journal of Chemotherapy. 1(2):113-22, 1989 Apr.
Abstract
Therapy of entamebiasis is critical in that, if untreated, the disease can be fatal. Recently, a new method for differentiating pathogenic and non-pathogenic amebae has been standardized. This method relies upon the electrophoretic analysis of 4 isoenzymes which allow the identification of 20 different zymodemes. It is now widely accepted that non-pathogenic strains of Entamoeba histolytica are not a hazard for humans and therefore don't need therapy. As a consequence, treatment must be addressed only toward infections caused by pathogenic strains. As there are different drugs available for treating amebiasis, from a therapeutical point of view the disease must be divided into two forms: intestinal and extraintestinal. For the former, drugs which reach therapeutical levels in the gut are required. The mainstay for the treatment of asymptomatic carriage of pathogenic strains is DILOXANIDE FUROATE, a very well tolerated luminal amebicide. METRONIDAZOLE and other 5-nitroimidazole compounds such as ORNIDAZOLE are indicated for the treatment of symptomatic intestinal infections as they reach good concentrations in tissues, including the bowel where ulcerations develop. In order to ensure the clearance of amebae from the gut, a subsequent cycle with diloxanide furoate is advisable. Extraintestinal forms include amebic abscesses which can develop in many sites, but most commonly in the liver. Metronidazole and related compounds are the drugs of choice; in case of liver abscess, the addition of CHLOROQUINE is indicated because of its good concentration in tissues. A subsequent cycle with diloxanide furoate is also indicated.(ABSTRACT TRUNCATED AT 250 WORDS)

Am. J. Trop. Med. Hyg., 28(2), 1979, pp. 190–193

SUCCESSFUL TREATMENT OF SYMPTOMATIC *ENTAMOEBA POLECKI* INFECTION*

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Abstract. The second symptomatic case of *Entamoeba polecki* infection, the first to respond to therapy, is reported. The patient experienced intermittent episodes of abdominal cramps, diarrhea, nausea, and malaise associated with large numbers of *E. polecki* cysts in the stool. Following treatment with diloxanide furoate and metronidazole, all symptoms cleared and the parasite was no longer present in the stool.

Human infection with *Entamoeba polecki* is rare and the parasite has been confused microscopically with *Entamoeba histolytica*.[1] It is generally considered not to be a pathogen for man. Herein, we report a case of prolonged symptomatic *E. polecki* intestinal disease in a patient in whom medical treatment for the organism resulted in the first clinical and laboratory cure. This is the 20th human case reported, the second in which the patient had been symptomatic.

CASE REPORT

D. McK., a 24-year-old Peace Corps volunteer, had been stationed in Upper Volta between July 1974 and June 1976, living in the back-country in open huts into which local domestic animals would freely roam. Pertinent animal contact occurred with pigs as well as with a pet monkey. Before entering the Peace Corps, he had no prior history of gastrointestinal disease. However, while in Africa, he experienced multiple episodes of dysentery diagnosed as both amebic and bacillary. With each episode he was treated with appropriate therapy and obtained temporary symptomatic improvement.

When discharged from the Peace Corps in June 1976 he again experienced abdominal pain and diarrhea and was successfully treated for hookworm infection. Nevertheless, he continued to have mucoid stools, diarrhea, nausea, headache, weakness, malaise, and abdominal cramps. His weight, which had been 155 lbs in June 1974, was 134 lbs 2 years later.

In September 1976 the patient was first seen at the National Naval Medical Center for investigation of his continued gastrointestinal complaints. At that time his physical examination was unremarkable, other than mucoid stool on rectal exam. A stool specimen observed for parasites showed a heavy infection with *Entamoeba polecki* and he had a 26% eosinophilia (Fig. 1).

The patient was begun on a course of metronidazole, 750 mg three times per day for 10 days and diiodohydroxyquin, 650 mg three times per day for 20 days. Although subjective improvement occurred and the parasite was absent from the stool briefly, both *E. polecki* cysts and his symptoms recurred (Fig. 2). Over the next 10 months the patient received numerous courses of treatment with anti-amebicides, with no sustained resolution of either symptoms or presence of the organism in his stool specimens, although symptomatic improvement often coincided with reductions in numbers of cysts in the feces during therapy. Extensive laboratory investigations seeking another cause of his diarrhea and or eosinophilia were normal or negative and are listed in Table 1. All routine laboratory tests were normal. A total of 31 stool specimens were examined with no other intestinal parasites observed. *Schistosoma haematobium* were not present in the urine. Repeated thick blood films did not show filariae. However, eosinophilia was always present and although it diminished after successful therapy for the *E. polecki*, it persisted (Figs. 1 and 2).

In July 1977, the luminal agent, diloxanide

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* The opinions or assertions contained herein are the private ones of the authors and not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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FIGURE 1. Representative *E. polecki* cysts. The karyosome is large in comparison with the nucleus and chromatin is abundant in the nuclear membrane. Chromatoid bodies are numerous (long arrow) while inclusion masses (short arrows) are seen in some cysts. Lawless and trichrome stain, × 960.

furoate (Furamide®), was used for the first time. Following a 10-day course of metronidazole 750 mg three times per day, diloxanide furoate was given for 10 days, 500 mg three times daily. Upon completion of taking both agents, he noticed subjective improvement in his condition with resolution of abdominal cramps, more formed, less mucoid stools and improved appetite. Although he had a rare isolated stool negative for parasites associated with treatment in the prior 10 months, he has subsequently had 12 consecutive negative stool exams over the past 12 months and only rarely has a loose stool following a dietary indiscretion, e.g., excessive beer consumption.

DISCUSSION

Entamoeba polecki was first described and named by von Prowazek in 1912 when it was first seen by him in pigs and later monkeys, cattle, and sheep, and was named after Dr. Poleck, a Samoan physician.[2] The life cycle includes both trophozoite and cyst stages, although trophozoites are infrequently seen in the stool. Almost exclusively described as a parasite of pigs and monkeys, it has been found in human stools on rare occasions.[1,3-6] Transmission from certain domestic animals, particularly pigs and monkeys, is the most likely source of infection, but human-to-human spread has also been suggested.[6]

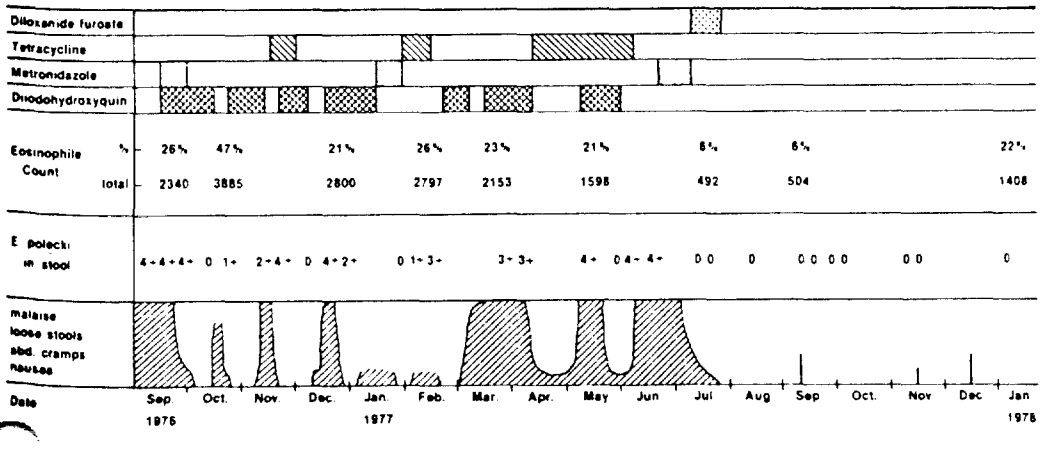

FIGURE 2. Course of illness showing symptoms, *E. polecki* cysts in stools, eosinophil count, and treatment. The patient remains asymptomatic and parasite free through June 1978.

TABLE 1

Laboratory studies performed to detect a potential cause of diarrhea and eosinophilia other than Entamoeba polecki. *All had normal results*

Serology*
Amebic IHA (× 4)
Schistosomal CFT and IFA
Trichinella LA slide test
Filarial BFT and IHA
Procedures
Sigmoidoscopy (× 2)
Rectal biopsy (histological and press exams)
Barium enema
Gall bladder series
Duodenoscopy and small intestinal biopsy
Duodenal aspirate examination
Upper gastrointestinal series with small bowel follow through

* IHA, indirect hemagglutination test; CFT, complement-fixation test; IFA, indirect fluorescent antibody test; LA, latex agglutination; BFT, bentonite flocculation test.

Differentiation between cysts of *E. histolytica*, *E. polecki*, and *E. coli* can be difficult.[1, 7] There are five major points of distinction between *E. histolytica* and *E. polecki*, the two amebae most often confused. First, *E. polecki* has a single nucleus with only about 1% of cysts reaching a binucleate stage. *E. histolytica* is infrequently uninucleate, and usually more mature cysts are seen containing 2–4 nuclei. The presence of only single nuclear forms in the stool should raise the suspicion of *E. polecki*. Second, the nucleus in the cyst of *E. polecki* is usually one-fourth to one-third the cyst's diameter and contains a large karyosome with variations of the chromatin pattern. In contrast, the *E. histolytica* nucleus is larger, being one-third to one-half the cyst's diameter, with a small karyosome and uniform distribution of peripheral nuclear chromatin. Third, *E. polecki* cysts rarely have glycogen vacuoles which are commonly seen with *E. histolytica*. However, an inclusion mass—a darkly stained body 3–4 times the size of the nucleus—is often found in the cytoplasm of *E. polecki*. It is not found in *E. histolytica*. Fourth, *E. histolytica* usually contains less than ten chromatin bars; *E. polecki* may have as many as thirty. Finally, *E. histolytica* is readily treatable; whereas, *E. polecki* is virtually refractory to therapy.[8] *E. polecki* is not invasive beyond the intestine; whereas, *E. histolytica* is well known for extra-intestinal complications.

This is only the second recorded case of [illegible] tomatic illness secondary to *E. polecki* intes[illegible] infection. Levin and Armstrong reported a [illegible] year-old female Peace Corps volunteer statio[illegible] in India with documented infestation for 12 months, the last 7 months away from the presumed contact area in India.[1] Our patient persisted in having uncontrolled symptomatic *E. polecki* intestinal disease for 10 months after leaving Africa until he was finally successfully treated. Furthermore, his diarrhea during the 2 years in Africa could have been at least in part due to *E. polecki* infection.

This infection has never previously been successfully eradicated from a human host. After 9 months of follow-up, with 12 negative stool examinations, we consider the patient's infection to be cured. To our knowledge, this is the first time that diloxanide furoate had been used specifically to treat *E. polecki* infection. It is used as a luminal amebicide, and when combined with metronidazole is considered an ideal regimen for acute amebic dysentery.[9, 10] Side effects are virtually nonexistent with the exception of increased flatulence. It is also useful to eliminate cyst carriers and as an adjunct to metronidazole in treating amebic liver abscesses.[11] It is not known whether diloxanide furoate alone is sufficient to cure *E. polecki* infections. A combination with metronidazole, as used to treat our patient, may be required.

We found no explanation for this patient's persistent eosinophilia. Blood smears and serological tests for both filariae and trichinosis were negative, as were repeated stool examinations for other intestinal parasites and a duodenal aspirate looking for *Strongyloides stercoralis*. There was no clinical or laboratory evidence for a collagen vascular disease, allergies or leukemia.

ACKNOWLEDGMENT

Dr. Martin Wolfe of the Department of State supplied the diloxanide furoate and reviewed the manuscript.

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Nondysenteric Intestinal Amebiasis

Treatment With Diloxanide Furoate

Martin S. Wolfe, MD

Diloxanide furoate, an investigational, amebicidal drug in the United States, was given in a ten-day course of 500 mg three times a day. This therapy led to parasitologic and symptomatic cure in all 12 asymptomatic cyst carriers and in 52 of 65 patients with nondysenteric, symptomatic, intestinal amebiasis who had all contracted *Entamoeba histolytica* infections while abroad. Criteria for cure were the absence of *E histolytica* parasites in three complete stool examinations, one and three months following completion of treatment, and complete or marked symptomatic improvement. Excessive flatulence was a common, but the only significant side effect. The high effectiveness (83% cure rate), relative ease of administration, and minimal toxicity of diloxanide furoate indicates that this drug has numerous advantages over other primarily luminal-acting amebicides presently available in this country for the treatment of chronic amebiasis.

More than 90% of the individuals seen in our Tropical Medicine Unit, who have contracted amebiasis while traveling or living abroad, had an asymptomatic or a nondysenteric chronic or subacute form of infection. Similar findings are also reported in returnees to England and France.[1,2] It is much more unusual in these countries to see the fulminant dysenteric form of amebiasis in travelers, with its typical presentation of frequent bloody stools, fever, marked abdominal cramps, tenesmus, and weight loss. A spectrum of symptoms is seen with nondysenteric *Entamoeba histolytica* infections, ranging from asymptomatic and mildly symptomatic individuals with complaints of increased number of soft stools, intermittent constipation, excessive distention and flatulence, and increased fatigue to more severely infected individuals who do not have frank amebic dysentery, but show evidence of some invasion of the bowel wall as manifested by very frequent watery to mushy stools, lower abdominal cramps, weight loss, anorexia and nausea, and marked asthenia. The asymptomatic and mildly symptomatic individuals fit most definitions of so-called chronic amebiasis, while the more severely affected individuals could be said to have a subacute type of infection. The present report concerns 100 individuals exposed to amebiasis while living or traveling outside the United States and found, upon their return to Washington, DC, to have cysts larger than 10μ, or trophozoites fitting the definition for *E histolytica*. These people were treated with diloxanide furoate (Furamide).

A number of anilides looked promising as amebicides and one of them, dichloroacet - 4 - hydroxy - *N* - methylanilide, was selected for further investigation. Ultimately, after extensive laboratory and clinical investigations, it was marketed in Britain under the name of Entamide and received the approved name diloxanide. Further investigations were carried out to find a derivative of diloxanide which would give better results in acute as well as in chronic amebiasis. Of the many derivatives examined, the furoate ester not only gave better results in acute amebic dysentery, but in the asymptomatic condition as well; it also was less toxic than diloxanide itself. This compound was given the name diloxanide furoate. It is less soluble than its earlier parent compound and is more slowly absorbed from the bowel and excreted from the body, thus providing a higher concentration in the bowel wall and lumen for a longer period of time. Diloxanide furoate has been used extensively outside of the United States for more than 15 years in the treatment of amebiasis, but has not been licensed in this country and is restricted by federal law to investigational use. Early studies carried out in various parts of the world with diloxamide furoate used by itself, both in indigenous populations in highly endemic amebiasis areas, and to a lesser extent in returnees from amebiasis-endemic areas to England and France, have claimed cure rates of more than 90% in the chronic forms of amebiasis and cure rates in the range of 80% in the treatment of acute amebic dysentery.[1-3] However, diloxanide furoate gave only a 40% cure rate and was considered inadequate treatment for acute amebic dysentery encountered in Durban, South Africa.[4] Other workers also consider diloxanide furoate inferior to other better-absorbed drugs in acute amebic dysentery where there is significant tissue invasion.[5,6]

The only previous studies of diloxanide furoate in the United States were by McHardy in 1960 who reported a 90% cure rate in asymptomatic amebiasis patients (Panel on Diarrheal Disease, clinical meeting of the American Medical Association, Washington, DC); and Most (written communication, March 1970), who used diloxanide furoate both with and without chloroquine phosphate and had cure rates between 75% and 80%. Neither of these investigators reported significant toxicity.

With its reported high effectiveness, relative ease of administration, and minimal toxic effects, diloxanide furoate appeared to have numerous advantages over other primarily luminal-acting amebicides presently available in the United

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Table 1.—Results*

Follow-Up	No. Treated	Cured†	
		No.	%
Complete			
Asymptomatic cases	12	12	100
Symptomatic cases	65	52	80
Total	**77**	**64**	**83**
Incomplete (eliminated)			
Asymptomatic cases	6	...	...
Symptomatic cases	17	...	...
Total	**23**		
Grand total	**100**		

*One hundred cases treated with diloxanide furoate.
†Based on three negative results from complete stool examinations at one and three months after treatment (a total of six negative stools) and a complete or marked symtomatic improvement.

Table 2.—Pretreatment and Posttreatment Symptoms*

Symptom	Pretreatment		Posttreatment	
	Cured (52)	Failures† (13)	Cured (52)	Failures† (13)
Anorexia	7	2	0	0
Nausea	6	2	0	0
Weight loss	10	1	0	0
Mushy stools	40	8	11	4
Watery stools	1	1	0	0
Mucus	4	1	0	0
Intermittent constipation	11	2	0	2
Abdominal cramps	12	3	3	0
Distention	16	5	1	2
Flatulence	31	8	5	4
Fatigue	17	5	3	1
At least one posttreatment symptom			17	8

*Sixty-five symptomatic cases with complete follow-up. Twelve asymptomatic cases not included.
†Based on the persistence of *E histolytica* parasites in posttreatment stool specimens.

States for the treatment of nondysenteric forms of amebiasis. A new drug investigation application was filed with the Food and Drug Administration for diloxanide furoate, and the study to be described was carried out from June 1970 through December 1971.

Materials and Methods

The 100 individuals in the study included 84 US foreign service employees or their dependents; all but five of these were adults, a reflection of the primarily adult population seen in the Tropical Medicine Unit of the Department of State Office of Medical Services. A further 16 adults were employees of the World Bank Group, comprising various nationalities, who reside in Washington, DC, but make frequent short trips to countries in the developing world. Eighteen patients were asymptomatic and the remaining 82 had mild to moderate symptoms from their infection.

Criteria for inclusion in the study included the finding of *E histolytica* cysts or trophozoites or both in at least one pretreatment stool examination; the absence of severe symptoms or proctoscopic findings compatible with acute amebic dysentery; and an expectation that the patient would remain in Washington, DC, during the three-month follow-up period. However, the nature of the patients' work required sudden travel to amebiasis endemic areas during this follow-up period in some cases. Informed consent for use of an investigational drug was obtained from all patients. Pretreatment and posttreatment proctoscopic examinations were not routinely carried out.

The criterion for parasitologic cure was the absence of *E histolytica* parasites in three stool examinations carried out one and three months following the completion of treatment, for a total of six negative specimens. All stool examinations were performed by the parasitology laboratory of the Department of State Office of Medical Services. Each examination consisted of direct smears in saline and iodine, zinc sulfate, and formaldehyde-ether concentration, and amebic culture utilizing liver-cholesterol broth. Dobell's hematoxylin-stained slides were made from any specimen containing suspicious parasites. In some cases, when patients had been suddenly reassigned or were abroad at the time of follow-up, specimens were sent to our laboratory in thimerosal-iodine-formaldehyde preservative, and direct smear and concentration technique were performed on these specimens.[7] Complete or marked symptomatic improvement was also necessary to consider an individual to be cured.

All subjects were treated on an outpatient basis. Adults were given a course of 500 mg of diloxanide furoate three times daily for ten days and children were given 20 mg/kg of body weight daily in three divided doses for ten days.

Pretreatment and immediate posttreatment white blood cell count, hematocrit reading, and urine albumin and sediment examinations were performed by the clinical laboratory of the Department of State Office of Medical Services. Following the completion of the treatment course, patients were questioned as to the occurrence of side effects and improvement in symptoms.

Results

Twenty-three patients did not have satisfactory results of posttreatment follow-up stool examinations and were eliminated from the study. Six of these were asymptomatic and the other 17 were symptomatic.

All 12 of the asymptomatic patients who had complete follow-up had six negative results for stool examinations and were considered parasitologically cured.

Sixty-five symptomatic patients had complete follow-up and 52 of these were considered parasitologically cured and symptomatically improved, a cure rate of 80% in this group (Table 1). Of the 13 parasitologic failures, five had complete symptomatic improvement, while at least one pretreatment symptom persisted in the remaining eight. Nine of the 13 treatment failures were manifested at the one-month follow-up examination and two of those found

positive at the three-month follow-up examination had traveled to amebiasis-endemic areas between the one- and three-month follow-up examinations. Six patients with treatment failure were given a second course of diloxanide furoate and three were cured, but *E histolytica* infection persisted in the other three. These second courses of diloxanide furoate are not included in determining cure rates.

The total cure rate obtained in the 77 patients (12 asymptomatic and 65 symptomatic) with satisfactory follow-up findings was 83% (Table 1).

In the successfully treated symptomatic group, all those with symptoms of anorexia, nausea, constipation, and weight loss, were free from these symptoms following treatment. Eleven individuals with soft stools before treatment continued to have soft stools during follow-up. Five of the 31 individuals who had had excessive flatulence still had this complaint after treatment, but only one of 16 individuals who had complained of distention continued to have this disturbance. Fatigue persisted in only three of 17 people who had had this complaint (Table 2).

Twenty-six patients who had complete follow-up had coincidental *Entamoeba hartmanni* infections before treatment, and in only two of them was this parasite present in posttreatment specimens.

A universal side effect was excessive flatulence, and 87% of those questioned as to occurrence of side effects complained of this. The only other significant side effects occurred in five patients who complained of nausea, three of anorexia, two of diarrhea, and two of mild abdominal cramps while taking the drug, but all completed the full course of treatment without incident (Table 3). No significant abnormalities were found between pretreatment and posttreatment blood cell counts and urinalyses.

Table 3.—Side Effects*

	Recorded	%
Flatulence	78	87.4
Anorexia	3	3.3
Nausea	5	5.6
Diarrhea	2	2.2
Abdominal cramps (mild)	2	2.2

*One hundred patients treated with diloxanide furoate. Ninety were followed up and recorded and ten were in a group with no or an incomplete follow-up.

Comment

The results of this investigation, the elimination of cysts from all 12 asymptomatic patients, and an overall cure rate of 83% in the 77 diloxanide furoate-treated patients with adequate follow-up, are similar to those of the two previous studies of this drug in the United States by McHardy and Most (written communication, March 1970). The results are also considered comparable to those obtained in other investigations of diloxanide furoate in nonendemic amebiasis areas. In one of the early studies of diloxanide furoate at the Hospital for Tropical Diseases in London, a 95.5% cure rate was obtained in 35 patients with chronic amebiasis when given a standard ten-day course of diloxanide furoate. However, in that study the mean number of follow-up stool examinations was only 1.5[6]; and it is quite likely that if six follow-up examinations over a three-month period had been carried out as in the present study, the cure rate would have dropped somewhat. In another study, Felix et al[2] treated 54 young adults with chronic amebiasis, who had returned to France after having spent a period in Algeria, with a standard course of diloxanide furoate. Cure was claimed for all but two of these patients, but follow-up, for the most part, was accomplished for only a fortnight after the end of treatment.[2] Other reported trials showing a high cure rate with diloxanide furoate were carried out primarily on residents in highly endemic amebiasis areas,[7] and are not thought to be comparable to the present trial that was carried out in nonendemic areas where the disease manifestations differ and the risk of reinfection during follow-up is minimal.

The good result with only minor attendant side effects obtained in this study with a ten-day course of diloxanide furoate compares favorably with and in many respects is superior to other amebicides used in the United States and elsewhere in the treatment of nondysenteric amebiasis. In England, orally given emetine-bismuth-iodide is suggested as the standard of reference against other amebicides in drug trials,[1] but this drug is not available in the United States and frequent troublesome side effects can occur with its use. Tetracyclines, particularly oxyetetracycline (Terramycin), and tetracycline hydrochloride, usually given in a dose of 1 or 2 gm daily for ten days, are highly effective in acute amebic dysentery, but relapse rates are high.[8] However, when this course is combined with or followed by a 21-day course of 650 mg of diiodohydroxyquin (Diodoquin) three times a day (often this course is combined or followed with a four-week course of chloroquine phosphate to prevent later development of liver abscess), cure rates as high as 95% without relapse have been obtained in acute amebic dysentery.[9] There are no valid reports on the effectiveness of this combined regimen in the more chronic forms of amebiasis, but cure rates of 80% to 90% should be obtainable. However, this regimen has numerous drawbacks, including (1) a prolonged course of treatment, (2) the not infrequent occurrence of diarrhea, (3) the potential risk of bacterial and monilial overgrowth, (4) teeth discoloration in children due to the tetracycline; and (5) the potential for reactions to iodine with diiodohydroxyquin. Diiodohydroxyquin, by itself in a 21-day course, is usually well tolerated and provides cures on the order of at least 75% in chronic amebiasis,[10] but again there is a rather prolonged course of treatment and iodine-sensitive individuals cannot use it. Also, this drug, though to a far lesser degree than the related compound iodochlorhydroxyquin (Entero-Vioform),[11] has led to a few cases of optic atrophy or polyneuropathy or both with long-term administration of larger doses than recommended for treatment of amebiasis, such as in the management of acrodermatitis enteropathica.[12] Paromomycin (Humatin) can be given in a short course and cure rates averaging 80% have been reported, but it is more effective in acute intestinal amebiasis than in the asymptomatic carrier state.[13] When it is used alone, relapses are frequent, and in the required doses, it frequently causes diarrhea and other gastrointestinal complaints and can lead to a reversible malabsorption defect.[14] Carbarsone, an arsenical, when employed alone is curative in only

about 50% of cases.[8] Although it is normally well tolerated, fatalities as a result of exfoliative dermatitis, liver necrosis, or hemorrhagic encephalitis have been reported.[15] Glycobiarsol (Milibis), is also an arsenical and when given alone, the cure rate is disappointingly low.[16] Although side effects are fewer with glycobiarsol than with carbarsone, isolated instances of arsenical toxicity have been reported.[16] In view of the wide range of amebicides available, it is doubtful whether arsenicals should be used for a chronic condition. Metronidazole has been heralded as the most effective drug for all forms of amebiasis. Although metronidazole has been shown to be highly effective in acute invasive forms of amebiasis,[17] claims that it is superior to primarily luminal-acting drugs, when used by itself in noninvasive forms of amebiasis, are not substantiated in all studies. Quite favorable results were obtained by the use of metronidazole alone in various dosage regimens in Pakistan[18] and India.[19] But studies in Bangkok[20] and London[21] showed metronidazole by itself to be much less satisfactory for the treatment of noninvasive forms of amebiasis in the lumen of the bowel. In the London study, carried out at the Hospital for Tropical Diseases, it was concluded that the cure rate in these forms of amebiasis was no higher with metronidazole in adequate doses than with diloxanide furoate alone, and side effects were more common and troublesome. It was thought that metronidazole is less effective as a luminal amebicide because it is almost completely absorbed from the small bowel and may thus only affect intraluminal amebae if they are in very close proximity to the colonic mucosa.[21]

A two- to four-week course of chloroquine phosphate, concomitant with or following the use of some of the drugs discussed, is often employed as a precautionary measure against the possible subsequent occurrence of amebic liver abscess.[22] The later development of amebic liver abscess is a rare occurrence in well-nourished, otherwise healthy individuals with nondysenteric amebiasis who comprised the subjects of the present study. Since side effects of chloroquine phosphate are frequent and may be additive to those caused by primary bowel-active drugs, such as diloxanide furoate (or tetracyclines, metronidazole, or diiodohydroxyquin used in other studies), and since we have not encountered amebic liver abscess following the use of these drugs, chloroquine phosphate has not been used as a routine precautionary measure in the treatment of the usual noninvasive amebiasis patient in our unit.

Little is known concerning possible teratogenic effects of diloxanide furoate, and since the main indication for its use is for a nonacute condition, it appears best at this time to withhold its use at least during the early stage of pregnancy. Some investigators have shown activated charcoal to be beneficial in decreasing the only common side effect of diloxanide furoate, excessive flatulence,[2] but this preparation was not used in the present study.

Although diloxanide furoate by itself may not be a satisfactory treatment for acute amebic dysentery,[4-6] it holds great promise as a luminal amebicide in a follow-up course of treatment to metronidazole in cases of acute amebiasis, particularly if diloxanide furoate becomes licensed and readily available for treatment of amebiasis in this country. This combination of metronidazole (in a dose of 500 to 750 mg three times a day for five to ten days) followed by a ten-day standard course of diloxanide furoate, could possibly represent a near ideal regimen for acute amebic dysentery and could also possibly lead to an even higher cure rate in nondysenteric amebiasis than either drug alone. It might also prove superior to the present commonly used regimen of a course of metronidazole followed by 21 days of diiodohydroxyquin therapy, which was recently reported to have been followed by the appearance of amebic liver abscesses in five patients whose amebic colitis had been successfully treated with these two drugs.[23] We are presently evaluating a regimen of metronidazole and diloxanide furoate in a series of patients with more acute signs and symptoms of amebiasis than those included in the present investigation, wherein diloxanide furoate by itself has been shown to be an effective, safe, and simple regimen for the treatment of chronic and subacute nondysenteric amebiasis.

Diloxanide furoate would therefore appear to be a valuable addition to the assortment of amebicidal drugs used in this country and it is hoped that it can be soon licensed for routine use.

Nonproprietary Name and Trademark of Drug

Metronidazole–*Flagyl*.

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Treatment of non-invasive amoebiasis. A comparison between tinidazole alone and in combination with diloxanide furoate

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Summary

Tinidazole (40 mg/kg body-weight in one daily dose for five days) and tinidazole (same dose) plus diloxanide furoate (20 mg/kg body-weight divided into three daily doses for 10 days) were compared as treatments for amoebiasis. The parasitic cure rates were 44 and 91% respectively. We cannot, therefore, recommend tinidazole alone in this dosage as a treatment for non-invasive amoebiasis.

Introduction

Tinidazole (Fasigyn) has recently been widely used as an alternative to metronidazole for the treatment of infections with *Entamoeba histolytica*. In a previous study (PEHRSON, 1982), tinidazole was given to a series of patients with chronic intestinal or asymptomatic amoebiasis. When checked by at least three stool specimens taken on different days, one month after treatment, we found a parasitic cure rate (p.c.r.) of 0% (0/14). This should be compared with the results obtained in other studies, showing a cure rate of 77 to 96% (MISRA & LAIQ, 1974; PRAKASH *et al.*, 1974; JOSHI & SHAH, 1975; BAKSHI *et al.*, 1978), using the same dosage schedule but mainly in cases of acute intestinal amoebiasis.

To investigate the reasons for the unsatisfactory response we obtained, which could be due to too low a dose or to a low efficiency of tinidazole in the gut lumen, we carried out a new trial with a higher daily dose of tinidazole and compared the effect of this higher dose with that following treatment with tinidazole and diloxanide furoate (Furamide) in combination. This latter was found to be an effective intraluminal amoebicide (WOODRUFF & BELL, 1960, 1967; WOLFE, 1973), whose mode of action upon the amoeba is unknown. We omitted Furamide as a single regimen, because it is considered to be ineffective against invasive amoebiasis and there is always a risk of developing an invasive form of the disease if zymodeme differentiation of strains of *Entamoeba histolytica* is not performed routinely (SARGEAUNT & WILLIAMS, 1978; SARGEAUNT *et al.*, 1982).

Materials and Methods

During the period of the study, 41 patients were diagnosed as suffering from amoebiasis. All of them were supposed to have contracted their infections abroad, as amoebiasis is not considered to be endemic in Sweden. No cases of acute, dysenteric amoebiasis or diagnosed or suspected cases of liver abscess were included. The patients had not received any anti-amoebic drug during the previous year. Nine of the patients had a concomitant infection with *Giardia lamblia*, two with *Shigella flexneri*, two with *Campylobacter jejuni*, one with *Salmonella paratyphi A*, one with *Hymenolepis nana*, one with *Ascaris lumbricoides* and one with *Trichuris trichiura*.

In a predetermined, random order, the patients were allocated to two groups, 18 being treated with tinidazole alone and 23 with the combination. All were hospital in-patients and kept under supervision during treatment.

Dosage schedules

(1) tinidazole 40 mg/kg body-weight in one daily dose for five days;

(2) tinidazole as above plus diloxanide furoate 20 mg/kg body-weight divided into three daily doses for 10 days.

Approximately one month after the treatment was completed, checks were made, including the examination of at least three stool specimens taken on different days. One of these was examined by direct microscopy of freshly passed, loose faeces induced by a 50% magnesium sulphate purgative and the other normally passed specimens were examined by the formol-ether-concentration technique described by RIDLEY & HAWGOOD (1956). Failure was defined as the persistence of amoebic trophozoites or cysts in any of these specimens.

Those in whom the treatment with tinidazole failed were later treated with the combination of tinidazole and diloxanide furoate and those in whom the combination failed were treated with metronidazole 40 mg/kg body-weight daily for 10 days.

Results

Data on the participants and the results of the checks one month after treatment are shown in Table I. In no case were the side effects severe enought to cause cessation of treatment. Statistical analysis was made, using the chi-square test, and showed a significant difference between the two groups on the 1%-level (two-tailed test) and in favour of the combination. No differences could be found between the response of Swedes and that of the immigrants, or between those infected on different continents (Asia, Africa, South America). The presence of other parasites did not seem to affect the outcome of the treatment.

Discussion

Our results with tinidazole alone (44% p.c.r.), in treating non-dysenteric amoebiasis, are unsatisfactory and differ very much from those obtained in previously published studies by different authors, using the same dosage schedules (77 to 96% p.c.r.) (ISLAM & HASAN, 1975; APTE & PACKARD, 1978) or lower (MISRA & LAIQ, 1974; PRAKASH *et al.*, 1974; JOSHI & SHAH, 1975; BAKASHI *et al.*, 1978). The patients in these studies were, however, mainly cases of acute amoebic dysentery, a factor which may have influenced the results.

A weak amoebicidal effect of the nitroimidazoles on the cyst stage of *E. histolytica* was observed by

Table I—Some characteristics and treatment results of 41 patients with non-invasive amoebiasis

Treatment	No.	Median age (age range) years	Patients with symptoms v. asymptomatics	Swedes v. other nationalities	Parasite-free at check	Parasite cure rate
Tinidazole 40 mg/kg × 1 + V	18	28 (9-68)	11:7	8:10	8	44%
Tinidazole 40 mg/kg × 1 × V + diloxanide furoate 500 mg × 3 × X	23	26 (6-68)	15:8	11:12	21	92%

SPILLMAN *et al.* (1976), but this report was contradicted by BAKSHI *et al.* (1978). Our drug trial was carried out in a country in which amoebiasis is not endemic, making reinfection during follow-up very unlikely, and confirming that the low p.c.r. was caused by "true" treatment failures.

We therefore believe that our poor results with tinidazole alone are due to its ineffectiveness in eradicating cysts in the lumen of the gut, either because of too effective absorption (MONRO, 1974) or inactivation by aerobic organisms as shown by RALPH & CLARKE (1978).

When tinidazole was combined with diloxanide furoate, we obtained a cure rate of 91%, which may be compared with studies by WOODRUFF & BELL (1967), in which they reported a cure rate of 95% in amoebic cyst-passers treated with diloxanide furoate alone for 10 days and WOLFE (1973), who found a cure rate of 83% using the same schedule. It is also noteworthy that all our failures with tinidazole alone have proved to be freed from their infection after treatment with the combination.

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Prakash, C., Bansal, B. C. & Bansal, M. R. (1974). Tinidazole in symptomatic intestinal amoebiasis. *Journal of Tropical Medicine and Hygiene*, 77, 165-167.

Ralph, E. D. & Clark, D. A. (1978). Inactivation of metronidazole by anaerobic and aerobic bacteria. *Antimicrobial Agents and Chemotherapy*, **14**, 377-383.

Ridley, D. S. & Hawgood, B. C. (1956). The value of formol-ether concentration of faecal cysts and ova. *Journal of Clinical Pathology*, **9**, 74-76.

Sargeaunt, P. G. & Williams, J. E. (1978). The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72**, 519-521.

Sargeaunt, P. G., Jackson, T. F. H. G. & Simjee, A. (1982). Biochemical homogeneity of *Entamoeba histolytica* isolates, especially those from liver abscess. *Lancet*, i, 1386-1388.

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Woodruff, A. W. & Bell, S. (1960). Clinical trials with entamide furoate and related compounds: I In a non-tropical environment. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **54**, 389-395.

Woodruff, A. W. & Bell, S. (1967). The evaluation of amoebicidies. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **61**, 435-439.

Accepted for publication 30th March, 1983.

A. INGREDIENT NAME:

FERRIC SUBSULFATE PURIFIED POWDER

B. Chemical Name:

Approximately $Fe_4(SO_4)_5(OH)_2$

C. Common Name:

D. Chemical grade or description of the strength, quality, and purity of the ingredient:

Feric Subsulphate (variable)

E. Information about how the ingredient is supplied:

Off white to pale yellow to brown fine powder, is odorless.

F. Information about recognition of the substance in foreign pharmacopeias:

The Pharmacopeia of the U.S.

G. Bibliography of available safety and efficacy data including peer reviewed medical literature:

H. Information about dosage forms used:

Topically

I. Information about strength:

J. Information about route of administration:

Topically

K. Stability data:

Decomposition: 520°C

L. Formulations:

M. Miscellaneous Information:

30-1328
54813

Please Refer to your P.O.# 54786 for the product listed below.

CHEMICAL NAME: FERRIC SUBSULFATE PURIFIED

CATALOG NUMBER: F1042

LOT NUMBER: LF0302

Seller certifies that the processes used in the manufacturing of the above items were in compliance with the applicable specifications as referred in or furnished with this purchase order.

Sincerely,

Lilian D. Casabar

12/97

LILIAN D. CASABAR
CofA COORDINATOR

enc /

QUALITY CONTROL REPORT

CHEMICAL NAME.: FERRIC SUBSULFATE POWDER A

MANUFACTURE LOT NO.: LF0302

PHYSICAL TEST

SPECIFICATION TEST STANDARD.:USP__/BP__/MERCK__/NF__/MART.__/CO.SPECS.__.

1)DESCRIPTION.:
E OFF WHITE TO PALE YELLOW TO BROWN FINE POWDER.IS ODORLESS.

2)SOLUBILITY.:
SLIGHTLY SOLUBLE IN WATER AND IN ALCOHOL.

3)MELTING POINT.:

4)SPECIFIC GRAVITY.:

5)IDENTIFICATION.:
A)A SOLUTION RESPONDS TO THE TESTS FOR FERRIC.

PASSES.:_____ **FAILS.:**_____

COMMENTS.:PRODUCT GETS AFFECTED BY LIGHT AND AIR.

ANALYST SIGNATURE.:_____ **DATE.:**_____

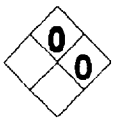
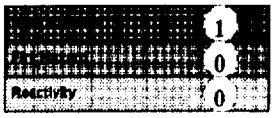
PREPACK TEST.:_____ **DATE.:**_____ **INITIAL.:**_____

RETEST.:_____ **DATE.:**_____ **INITIAL.:**_____

SPECTRUM®

GARDENA, CA
NEW BRUNSWICK, NJ

Material Safety Data Sheet

NFPA	HMIS	Personal Protective Equipment
		
		See Section 15.

Section 1. Chemical Product and Company Identification

Common Name/ Trade Name	**Ferric subsulfate**	**Code**	F3150
		CAS#	1310-45-8
Manufacturer	SPECTRUM CHEMICAL MFG. CORP. 14422 SOUTH SAN PEDRO STREET GARDENA, CALIFORNIA 90248	**RTECS**	Not available.
		TSCA	On the TSCA list.
Commercial Name(s)	Monsel's Salt	**CI#**	Not applicable.
Synonym	Basic ferric sulfate		
Chemical Name	Not available.		
Chemical Family	Salt.		
Chemical Formula	Fe4(CH)2(SO4)5		
Supplier	SPECTRUM QUALITY PRODUCTS, INC. 14422 SOUTH SAN PEDRO STREET GARDENA, CA 90248		

IN CASE OF EMERGENCY
CHEMTREC (24hr) 800-424-9300
Emergency phone: (310)516-8000

Section 2.Composition and Information on Ingredients

		Exposure Limits			
Name	**CAS #**	**TWA (mg/m³)**	**STEL (mg/m³)**	**CEIL (mg/m³)**	**% by Weight**
Ferric subsulfate	1310-45-8	1			100

Toxicological Data on Ingredients

Ferric subsulfate
LD50: Not available.
LC50: Not available.

Section 3. Hazards Identification

Potential Acute Health Effects

Slightly dangerous to dangerous in case of ingestion. Very slightly to slightly dangerous in case of eye contact (irritant), of inhalation.

Potential Chronic Health Effects

Very slightly to slightly dangerous in case of eye contact (irritant), of inhalation.
CARCINOGENIC EFFECTS: Not available. **MUTAGENIC EFFECTS**: Not available. **TERATOGENIC EFFECTS**: Not available. The substance is toxic to blood, kidneys, liver. Toxicity of the product to the reproductive system: Not available. Repeated or prolonged exposure to the substance can produce target organs damage.

WARNING: This product contains a chemical known to the State of California to cause cancer.
Chemical ingredient(s) requiring this warning:

NONE

WARNING: This product contains a chemical known to the State of California to cause birth defects or other reproductive harm.
Chemical ingredient(s) requiring this warning:

NONE

Continued on Next Page

Section 4. First Aid Measures

Eye Contact	IMMEDIATELY flush eyes with running water for at least 15 minutes, keeping eyelids open. COLD water may be used
Skin Contact	NO known EFFECT according to our database.
Serious Skin Contact	No additional information.
Inhalation	Allow the victim to rest in a well ventilated area. Seek immediate medical attention.
Serious Inhalation	No additional information.
Ingestion	Remove dentures if any. Have conscious person drink several glasses of water or milk. INDUCE VOMITING by sticking finger in throat. Lower the head so that the vomit will not reenter the mouth and throat. NEVER give an unconscious person anything to ingest. Seek medical attention.
Serious Ingestion	No additional information.

Section 5. Fire and Explosion Data

Flammability of the Product	Non-flammable.
Auto-Ignition Temperature	Not applicable.
Flash Points	Not applicable.
Flammable Limits	Not applicable.
Products of Combustion	Not applicable.
Fire Hazards in Presence of Various Substances	Not applicable.
Explosion Hazards in Presence of Various Substances	Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available. No specific information is available in our database regarding the product's risks of explosion in the presence of various materials
Fire Fighting Media and Instructions	Non-flammable.
Special Remarks on Fire Hazards	No additional remark.
Special Remarks on Explosion Hazards	No additional remark.

Section 6. Accidental Release Measures

Small Spill	Use appropriate tools to put the spilled solid in a convenient waste disposal container. If necessary: **Neutralize the residue with a dilute solution of sodium carbonate.** Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements.
Large Spill	Our database contains no additional information in case of a spill and/or a leak of the product. Use a shovel to put the material into a convenient waste disposal container. **Neutralize the residue with a dilute solution of sodium carbonate.** Finish cleaning by spreading water on the contaminated surface and allow to evacuate through the sanitary system.

Section 7. Handling and Storage

Precautions	No specific safety phrase has been found applicable for this product.
Storage	No specific storage is required. Use shelves or cabinets sturdy enough to bear the weight of the chemicals. Be sure that it is not necessary to strain to reach materials, and that shelves are not overloaded.

Continued on Next Page

Section 8. Exposure Controls/Personal Protection

Engineering Controls	Use process enclosures, local exhaust ventilation, or other engeneering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit
Personal Protection	Safety glasses. Lab coat.
Personal Protection in Case of a Large Spill	Splash goggles. Full suit. Boots. Gloves. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.
Exposure Limits	TWA: 1 (mg/m^3) from OSHA/NIOSH [1993] TWA: 1 (mg/m^3) from ACGIH [1993] Consult local authorities for acceptable exposure limits.

Section 9. Physical and Chemical Properties

Physical state and appearance	Solid.	**Odor**	Not available.
Molecular Weight	Not available.	**Taste**	Strong.
pH (1% soln/water)	4	**Color**	Brown.
Boiling Point	Not available.		
Melting Point	Decomposes.		
Critical Temperature	Not available.		
Specific Gravity	Not available.		
Vapor Pressure	Not available.		
Vapor Density	Not available.		
Volatility	Not available.		
Odor Threshold	Not available.		
Water/Oil Dist. Coeff.	Not available.		
Ionicity (in Water)	Not available.		
Dispersion Properties	See solubility in water.		
Solubility	Easily soluble in cold water, hot water.		

Section 10. Stability and Reactivity Data

Stability	The product is stable.
Instability Temperature	Not available.
Conditions of Instability	No additional remark.
Incompatibility with various substances	No specific information is available in our database regarding the reactivity of this material in presence of various other materials.
Corrosivity	Non-corrosive in presence of glass.
Special Remarks on Reactivity	No additional remark.
Special Remarks on Corrosivity	No additional remark.
Polymerization	No.

Continued on Next Page

Section 11. Toxicological Information

Routes of Entry	Ingestion.
Toxicity to Animals	LD50: Not available. LC50: Not available
Chronic Effects on Humans	The substance is toxic to blood, kidneys, liver. Toxicity of the product to the reproductive system: Not available.
Other Toxic Effects on Humans	Slightly dangerous to dangerous in case of ingestion. Very slightly to slightly dangerous in case of eye contact (irritant), of inhalation.
Special Remarks on Toxicity to Animals	No additional remark.
Special Remarks on Chronic Effects on Humans	No additional remark.
Special Remarks on other Toxic Effects on Humans	No additional remark.

Section 12. Ecological Information

Ecotoxicity	Not available.
BOD5 and COD	Not available.
Products of Biodegradation	Some metallic oxides.
Toxicity of the Products of Biodegradation	The products of degradation are as toxic as the original product.
Special Remarks on the Products of Biodegradation	No additional remark.

Section 13. Disposal Considerations

Waste Disposal	Recycle to process, if possible. Consult your local or regional authorities.

Section 14. Transport Information

DOT Classification	Not a DOT controlled material (United States).
Identification	Not applicable (PIN and PG).
Special Provisions for Transport	Not applicable.
DOT (Pictograms)	

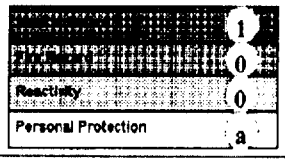
Section 15. Other Regulatory Information and Pictograms

Federal and State Regulations	The following product(s) is (are) listed on TSCA: **Ferric subsulfate**
California Proposition 65 Warnings	**WARNING: This product contains a chemical known to the State of California to cause cancer.** **Chemical ingredient(s) requiring this warning:** **NONE** **WARNING: This product contains a chemical known to the State of California to cause birth defects or other reproductive harm.** **Chemical ingredient(s) requiring this warning:** **NONE**
Other Regulations	OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).

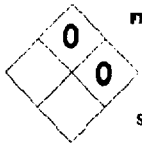
Continued on Next Page

Other Classifications	**WHMIS (Canada)** Not controlled under WHMIS (Canada).
	DSCL (EEC) Not controlled under DSCL (Europe)

HMIS (U.S.A.)



National Fire Protection Association (U.S.A.)



WHMIS (Canada) (Pictograms)

DSCL (Europe) (Pictograms)

TDG (Canada) (Pictograms)

ADR (Europe) (Pictograms)

Protective Equipment

Lab coat.

Safety glasses.

Section 16. Other Information

Catalog Number(s)	**F1042**
References	Not available.
Other Special Considerations	No additional remark.

Validated by E. Brull on 9/26/97.

Verified by E. Brull.

Printed 9/29/97.

Emergency phone: (310)516-8000

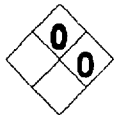
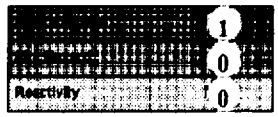
Notice to Reader

All chemicals may pose unknown hazards and should be used with caution. This Material Safety Data Sheet (MSDS) applies only to the material as packaged. If this product is combined with other materials, deteriorates, or becomes contaminated, it may pose hazards not mentioned in this MSDS. It shall be the user's responsibility to develop proper methods of handling and personal protection based on the actual conditions of use. While this MSDS is based on technical data judged to be reliable, Spectrum Quality Products, Inc. assumes no responsibility for the completeness or accuracy of the information contained herein.

SPECTRUM®

GARDENA, CA
NEW BRUNSWICK, NJ

Material Safety Data Sheet

NFPA	HMIS	Personal Protective Equipment
		
		See Section 15.

Section 1. Chemical Product and Company Identification

Common Name/ Trade Name	**Ferric Subsulfate Solution**
Manufacturer	SPECTRUM CHEMICAL MFG. CORP. 14422 SOUTH SAN PEDRO STREET GARDENA, CALIFORNIA 90248
Commercial Name(s)	Monsel's Solution
Synonym	Not available.
Chemical Name	Not applicable.
Chemical Family	Salt.
Chemical Formula	Not applicable.
Supplier	SPECTRUM QUALITY PRODUCTS, INC. 14422 SOUTH SAN PEDRO STREET GARDENA, CA 90248

Code	F3155
CAS#	Not applicable.
RTECS	Not applicable.
TSCA	All the ingredients are on the TSCA list
CI#	Not applicable.

IN CASE OF EMERGENCY
CHEMTREC (24hr) 800-424-9300

Emergency phone: (310)516-8000

Section 2. Composition and Information on Ingredients

		Exposure Limits			
Name	**CAS #**	**TWA (mg/m³)**	**STEL (mg/m³)**	**CEIL (mg/m³)**	**% by Weight**
Ferric subsulfate	1310-45-8	1			20-22
Water	7732-18-5				78-80

Toxicological Data on Ingredients

Ferric subsulfate
LD50: Not available.
LC50: Not available.

Section 3. Hazards Identification

Potential Acute Health Effects

Very slightly to slightly dangerous in case of eye contact (irritant), of ingestion, of inhalation. Not dangerous in case of skin contact (non-corrosive for skin, non-irritant for skin, non-sensitizer for skin, non-permeator by skin).

Potential Chronic Health Effects

Very slightly to slightly dangerous in case of eye contact (irritant), of inhalation.
Not dangerous in case of skin contact (non-corrosive for skin, non-irritant for skin, non-sensitizer for skin, non-permeator by skin), of ingestion.
CARCINOGENIC EFFECTS: Not available. **MUTAGENIC EFFECTS**: Not available. **TERATOGENIC EFFECTS**: Not available. The substance is toxic to blood, kidneys, liver. Toxicity of the product to the reproductive system: Not available. Repeated or prolonged exposure to the substance can produce target organs damage.

WARNING: This product contains a chemical known to the State of California to cause cancer. Chemical ingredient(s) requiring this warning:

NONE

Continued on Next Page

WARNING: This product contains a chemical known to the State of California to cause birth defects or other reproductive harm.
Chemical ingredient(s) requiring this warning:

NONE

Section 4. First Aid Measures

Eye Contact	IMMEDIATELY flush eyes with running water for at least 15 minutes, keeping eyelids open. COLD water may be used.
Skin Contact	NO known EFFECT according to our database.
Serious Skin Contact	No additional information.
Inhalation	Allow the victim to rest in a well ventilated area. Seek immediate medical attention.
Serious Inhalation	No additional information.
Ingestion	Remove dentures if any. Have conscious person drink several glasses of water or milk. INDUCE VOMITING by sticking finger in throat. Lower the head so that the vomit will not reenter the mouth and throat. NEVER give an unconscious person anything to ingest. Seek medical attention.
Serious Ingestion	No additional information.

Section 5. Fire and Explosion Data

Flammability of the Product	Non-flammable.
Auto-Ignition Temperature	Not applicable.
Flash Points	Not applicable.
Flammable Limits	Not applicable.
Products of Combustion	Not applicable.
Fire Hazards in Presence of Various Substances	Not applicable.
Explosion Hazards in Presence of Various Substances	Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available. No specific information is available in our database regarding the product's risks of explosion in the presence of various materials.
Fire Fighting Media and Instructions	Non-flammable.
Special Remarks on Fire Hazards	No additional remark.
Special Remarks on Explosion Hazards	No additional remark.

Section 6. Accidental Release Measures

Small Spill	Dilute with water and mop up, or absorb with an inert DRY material and place in an appropriate waste disposal container. If necessary: **Neutralize the residue with a dilute solution of sodium carbonate.** Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements.
Large Spill	Our database contains no additional information in case of a spill and/or a leak of the product. Absorb with an inert material and put the spilled material in an appropriate waste disposal. **Neutralize the residue with a dilute solution of sodium carbonate.** Finish cleaning by spreading water on the contaminated surface and allow to evacuate through the sanitary system.

Continued on Next Page

Section 7. Handling and Storage

Precautions	No specific safety phrase has been found applicable for this product.
Storage	No specific storage is required. Use shelves or cabinets sturdy enough to bear the weight of the chemicals. Be sure that it is not necessary to strain to reach materials, and that shelves are not overloaded.

Section 8. Exposure Controls/Personal Protection

Engineering Controls	Provide exhaust ventilation or other engeneering controls to keep the airborne concentrations of vapors below their respective threshold limit value.
Personal Protection	Safety glasses. Lab coat. Gloves.
Personal Protection in Case of a Large Spill	Splash goggles. Full suit. Boots. Gloves. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.
Exposure Limits	**Ferric subsulfate** TWA: 1 (mg/m³) from OSHA/NIOSH [1993] TWA: 1 (mg/m³) from ACGIH [1993] Consult local authorities for acceptable exposure limits.

Section 9. Physical and Chemical Properties

Physical state and appearance	Liquid.
Molecular Weight	Not applicable.
pH (1% soln/water)	4

Odor	Slight.
Taste	Strong.
Color	Brownish-red.

Boiling Point	100°C (212°F)
Melting Point	Not available.
Critical Temperature	Not available
Specific Gravity	1.58 (Water = 1)
Vapor Pressure	17.535 mm of Hg (@ 20°C) based on data for: Water
Vapor Density	0.62 (Air = 1) based on data for: Water
Volatility	Not available.
Odor Threshold	Not available.
Water/Oil Dist. Coeff.	Not available.
Ionicity (in Water)	Not available.
Dispersion Properties	See solubility in water.
Solubility	Easily soluble in cold water, hot water.

Section 10. Stability and Reactivity Data

Stability	The product is stable.
Instability Temperature	Not available.
Conditions of Instability	No additional remark.
Incompatibility with various substances	No specific information is available in our database regarding the reactivity of this material in presence of various other materials.
Corrosivity	Non-corrosive in presence of glass.
Special Remarks on Reactivity	No additional remark.
Special Remarks on Corrosivity	No additional remark.
Polymerization	Not available

Continued on Next Page

Section 11. Toxicological Information

Routes of Entry	Ingestion.
Toxicity to Animals	LD50: Not available LC50: Not available
Chronic Effects on Humans	The substance is toxic to blood, kidneys, liver Toxicity of the product to the reproductive system: Not available.
Other Toxic Effects on Humans	Very slightly to slightly dangerous in case of eye contact (irritant), of ingestion, of inhalation Not dangerous in case of skin contact (non-corrosive for skin, non-irritant for skin, non-sensitizer for skin, non-permeator by skin).
Special Remarks on Toxicity to Animals	No additional remark.
Special Remarks on Chronic Effects on Humans	No additional remark.
Special Remarks on other Toxic Effects on Humans	No additional remark.

Section 12. Ecological Information

Ecotoxicity	Not available.
BOD5 and COD	Not available.
Products of Biodegradation	Some metallic oxides.
Toxicity of the Products of Biodegradation	The product itself and its products of degradation are not toxic.
Special Remarks on the Products of Biodegradation	No additional remark.

Section 13. Disposal Considerations

Waste Disposal	Recycle to process, if possible. Consult your local or regional authorities.

Section 14. Transport Information

DOT Classification	Not a DOT controlled material (United States).
Identification	Not applicable (PIN and PG).
Special Provisions for Transport	Not applicable.
DOT (Pictograms)	

Section 15. Other Regulatory Information and Pictograms

Federal and State Regulations	The following product(s) is (are) listed on TSCA: **Ferric subsulfate, Water**
California Proposition 65 Warnings	**WARNING: This product contains a chemical known to the State of California to cause cancer.** **Chemical ingredient(s) requiring this warning:** **NONE** **WARNING: This product contains a chemical known to the State of California to cause birth defects or other reproductive harm.** **Chemical ingredient(s) requiring this warning:** **NONE**
Other Regulations	OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).

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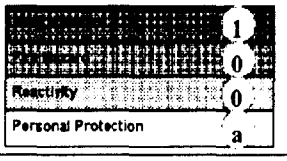
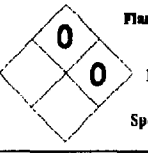
Other Classifications	**WHMIS (Canada)**	Not controlled under WHMIS (Canada).
	DSCL (EEC)	Not controlled under DSCL (Europe).

HMIS (U.S.A.)



National Fire Protection Association (U.S.A.)



WHMIS (Canada) (Pictograms)

DSCL (Europe) (Pictograms)

TDG (Canada) (Pictograms)

ADR (Europe) (Pictograms)

Protective Equipment

Gloves.

Lab coat.

Safety glasses.

Section 16. Other Information

Catalog Number(s)	FE107
References	Not available.
Other Special Considerations	No additional remark.
Validated by E. Brull on 9/26/97.	**Verified by E. Brull. Printed 9/29/97.**
Emergency phone: (310)516-8000	

Notice to Reader

All chemicals may pose unknown hazards and should be used with caution. This Material Safety Data Sheet (MSDS) applies only to the material as packaged. If this product is combined with other materials, deteriorates, or becomes contaminated, it may pose hazards not mentioned in this MSDS. It shall be the user's responsibility to develop proper methods of handling and personal protection based on the actual conditions of use. While this MSDS is based on technical data judged to be reliable, Spectrum Quality Products, Inc. assumes no responsibility for the completeness or accuracy of the information contained herein.

and titrate with 0.1 *N* potassium permanganate until a permanent pink color is produced. Each cc. of 0.1 *N* potassium permanganate corresponds to 15.19 mg. of $FeSO_4$. This assay is explained in the chapter on *Official Assays*.
Storage—Preserve the salt in well-closed containers.

Uses—This salt is more stable in air than the fully hydrated Ferrous Sulfate, and is more adaptable for making capsules, pills, and tablets.
Usual Dose—0.2 Gm. (approximately 3 grains).

Ferrous Sulfate Syrup U. S. P. Syrupus Ferri Sulfatis

[*Sp.* Jarabe de Sulfato Ferroso]

Ferrous Sulfate Syrup contains, in each 100 cc., not less than 3.75 Gm. and not more than 4.25 Gm. of $FeSO_4.7H_2O$.

	Metric	Alternative
Ferrous Sulfate	40 Gm.	1 oz. av. 147 gr.
Citric Acid	2.1 Gm.	31 gr.
Peppermint Spirit	2 cc.	31 min
Sucrose	825 Gm.	27 oz. av. 236 gr.
Distilled Water, a sufficient quantity,		
To make	1000 cc.	2 pints

Dissolve the ferrous sulfate, the citric acid, the peppermint spirit, and 200 Gm. of sucrose in 450 cc. of distilled water; and filter the solution until clear. Then dissolve the remainder of the sucrose in the clear filtrate, and add sufficient distilled water to make 1000 cc. Mix well and strain, if necessary, through a pledget of cotton.

Assay—Transfer 25 cc. of Ferrous Sulfate Syrup, accurately measured, to a 250-cc. Erlenmeyer flask. Add 15 cc. of diluted sulfuric acid and 100 cc. of water, and shake well. Titrate with 0.1 *N* ceric sulfate, using orthophenanthroline T.S. as the indicator. Each cc. of 0.1 *N* ceric sulfate is equivalent to 27.80 mg. of $FeSO_4.7H_2O$. This assay is explained in the chapter on *Official Assays*.
Storage—Preserve this Syrup in tight containers.

Uses—See *Ferrous Sulfate*.
Usual Dose—8 cc. (approximately 2 fluidrachms).

Ferrous Sulfate Tablets U. S. P. Tabellæ Ferri Sulfatis

[*Sp.* Tabletas de Sulfato Ferroso]

Ferrous Sulfate Tablets contain not less than 95 per cent and not more than 110 per cent of the labeled amount of $FeSO_4.7H_2O$. An equivalent amount of exsiccated ferrous sulfate may be used in place of $FeSO_4.7H_2O$ in preparing Ferrous Sulfate Tablets.

For tests for *Identification* and the *Weight variation* requirements, see the U. S. P.
Assay—Weigh a counted number of not less than 20 Ferrous Sulfate Tablets, and crush them well without appreciable loss. Weigh accurately in a beaker a portion of the crushed Tablets, equivalent to about 500 mg. of ferrous sulfate, and dissolve in a mixture of 20 cc. of diluted sulfuric acid and 80 cc. of freshly boiled and cooled water. Filter the solution rapidly as soon as all soluble ingredients in the tablets are dissolved, and wash the beaker and filter with small portions of a mixture of 20 cc. of diluted sulfuric acid and 80 cc. of water. Immediately titrate the combined filtrate and washings with 0.1 *N* ceric sulfate, using orthophenanthroline T.S. as the indicator. Each cc. of 0.1 *N* ceric sulfate is equivalent to 27.80 mg. of $FeSO_4.7H_2O$. This assay is explained in the chapter on *Official Assays*.
Storage—To minimize oxidation, these tablets should be kept in tight containers.

Usual Dose—0.3 Gm. (approximately 5 grains) of Ferrous Sulfate.

Unofficial Inorganic Iron Compounds

Ferric Ammonium Sulfate, Ferric Alum—An official reagent.
Ferric Chloride, Iron Perchloride [$FeCl_3.6H_2O$]—An official reagent.
Ferric Ferrocyanide, $Fe_4[Fe(CN)_6]_3$—Commonly called *Prussian blue*. A dark blue powder, insoluble in water. *Uses:* a pigment in paints, in inks, and in bluing. Formerly used in medicine as a tonic. *Dose:* 0.12 to 0.3 Gm. (2 to 5 grains).
Ferric Ferrocyanide, Soluble, Soluble Iron Ferrocyanide [approximately $KFe[Fe(CN)_6] + H_2O$]—A blue powder, soluble in water, forming a colloidal solution.
Ferric Fluoride [$FeF_3 + H_2O$]—White to slightly yellow, crystalline powder. Slightly soluble in water or in alcohol.
Ferric Hydroxide, Antidotum Fuchsi, Hydrated Ferric Oxide—See *Magma of Ferric Hydroxide* (page 248).
Ferric Nitrate [$Fe(NO_3)_3$]—Grayish white crystals; soluble in water. *Uses:* a mordant in dyeing and in calico-printing; also a tonic and astringent.
Ferric Oxide, Saccharated N. F. VII—The following directions were given:

> Dissolve 11 Gm. of monohydrated sodium carbonate in 150 cc. distilled water. Dilute 30 Gm. of ferric chloride solution with 150 cc. of distilled water and gradually add, with constant stirring, to the carbonate solution. Decant the supernatant liquid and wash the precipitate with distilled water until a portion of the washings, when diluted with 5 volumes of distilled water, give only a slight opalescence with silver nitrate T.S. Collect the precipitate on a cloth strainer, transfer the magma to a porcelain dish on a water bath, and add 50 Gm. of sucrose and a sufficient quantity (not more than 5 cc.) of a 15 per cent solution of sodium hydroxide to produce a clear solution. Evaporate the mixture to dryness upon a water bath and, if necessary, add sufficient powdered sucrose to make the product weigh 100 Gm. When so prepared it contains about 3 per cent of iron [Fe] corresponding to about 4.5 per cent of ferric oxide.

It occurs as a brown powder which is soluble in water but insoluble in alcohol. The iron in this preparation is non-ionic and it does not respond to some of the usual reactions for iron. *Uses:* a hematinic. *Dose:* 2 Gm. (30 grains) corresponding to 60 mg. of Fe.
Certain specially prepared solutions of saccharated ferric oxide may be administered intravenously, and are currently receiving trial for the treatment of refractory hypochromic anmeias.
Ferric Phosphate [$FePO_4.4H_2O$]—A nearly white or slightly yellow, crystalline powder. Insoluble in water or in acetic acid; soluble in mineral acid. *Uses:* source of iron for the enrichment of foods.
Ferric Pyrophosphate [$Fe_4(P_2O_7)_3.9H_2O$]—Nearly white to slightly yellow, crystalline powder. Insoluble in water; soluble in mineral acids; also soluble when freshly prepared, in an excess of alkali citrate, forming green solutions.
Ferric Pyrophosphate, Soluble—This is a complex salt of sodium ferricitropyrophosphate. It is made by the process described for *Soluble Ferric Phosphate*, replacing the sodium phosphate with sodium pyrophosphate. It contains 11 to 13 per cent of iron. The iron in this phosphate is, like that in Soluble Ferric Phosphate, non-ionic. It occurs as bright green scales or as granules. It is freely soluble in water but insoluble in alcohol. *Uses:* a hematinic. *Dose:* 0.25 Gm. (4 grains).
Ferric Subsulfate [approximately $Fe_4(SO_4)_5(OH)_2$]—Yellow, somewhat hygroscopic powder, very slowly and usually incompletely soluble in water; insoluble in alcohol. *Uses:* a styptic, and a mordant in textile dyeing.
Ferric Sulfate [$Fe_2(SO_4)_3$]—Grayish white, very hygroscopic powder. Slowly soluble in water, sparingly soluble in alcohol. *Uses:* employed in dyeing, in the manufacture of Prussian blue and inks, and in water purification.
Ferrous Ammonium Sulfate—An official reagent.
Ferrous Bromide [$FeBr_2$]—A yellowish, deliquescent crystalline powder. Soluble in water. *Uses:* alterative and tonic. *Dose:* 0.06 to 0.2 Gm. (1 to 3 grains).
Ferrous Chloride [$FeCl_2.H_2O$]—Pale green, deliquescent crystals, or crystalline powder. Oxidizes on exposure to air. Soluble in 1 part water acidulated with hydrochloric acid; incompletely soluble in alcohol. *Uses:* astringent in gargles; also a mordant in printing fabrics and in dyeing.
Ferrous Iodide [$FeI_2.4H_2O$]—Almost black, very deliquescent masses. Decomposes rapidly in air with liberation of iodine. Freely soluble in cold water, decomposed by hot water; also soluble in alcohol. *Uses:* an alterative and tonic, generally given in pills or capsules.
Ferrous Phosphate [$Fe_3(PO_4)_2.8H_2O$]—On account of rapid oxidation in air the article of commerce contains basic ferric phosphate. A grayish blue powder. Insoluble in water, soluble in mineral acid. *Uses:* a hematinic; also used in coloring ceramics. *Dose:* 0.3 to 0.5 Gm. (5 to 8 grains).
Ferrous Sulfide [FeS]—An official reagent.

Inorganic Iron Specialties

Note—The following preparations, containing iron per se, or iron supplemented with vitamins and other substances, are used as hematinics and dietary supplements. The dose varies with the requirements of the individual.

Aminoferin (J. T. Lloyd)—Liquid containing iron, aminoacetic acid, and thiamine hydrochloride in an oat menstruum.
Arsenoferratose (Rare-Galen)—Elixir, each fluidounce containing sodium ferrialbuminate sufficient to furnish 235 mg. iron, and 0.9 mg. arsenic; tablets, each containing sodium ferrialbuminate to furnish 16 mg. iron, and 0.09 mg. arsenic.
Ascoferrin (Dorsey)—Capsules, each containing 0.325 Gm. ferrous sulfate and 50 mg. ascorbic acid.
B Ferrated (Upjohn)—Elixir, each fluidounce containing 1.3 Gm. ferrous sulfate with vitamin B complex supplement.
Befolex (Central)—Tablets, each containing 19.4 mg. ferrous sulfate with folic acid and vitamin B complex factors.
Beofer (Rexall)—Elixir or tablets, each fluidounce of the elixir containing 3 gr. ferrous sulfate, 2 mg. riboflavin, and 1 mg. thiamine hydrochloride; each tablet containing 3 gr. ferrous sulfate, 5 mg. nicotinic acid, 0.08 mg. pyridoxine hydrochloride, 0.6 mg. riboflavin, and 120 U. S. P. units vitamin B_1.
Betacuron (Lakeside)—Liquid, each 30 cc. containing 274 mg. iron peptonate, 15.9 mg. copper gluconate, with vitamin B factors.
Betaferrum (Hart Drug)—Elixir or tablets, each fluidounce of elixir containing 20 gr. ferrous sulfate and 3.6 mg. thiamine hydrochloride; each tablet containing 3 gr. exsiccated ferrous sulfate and 1.0 mg. thiamine hydrochloride.
Betaron (Warren-Teed)—Syrup, each fluidounce containing 1 Gm. ferrous sulfate and 3 mg. thiamine hydrochloride.
Biatron (National Drug)—Elixir, each fluidounce containing 4 gr. green iron and ammonium citrates, 5 per cent alcohol, 4 gr. calcium

		O=16
Dithymol Diiodide (Thymol Iodide)	$(C_{10}H_{12}O)_2I_2$	550.03
Dysprosium	**Dy**	**162.5**
Emetine	$C_{15}H_{22}O_2N$	248.19
" Hydrochloride, Anhydrous	$C_{30}H_{44}O_4N_2.2HCl$	569.31
Epinephrine, Hydrated	$C_9H_{13}O_3N+\frac{1}{2}H_2O$	192.12
Erbium	**Er**	**167.7**
Erythrol Tetranitrate	$C_4H_6(NO_3)_4$	302.09
Ether (Ethyl Oxide)	$(C_2H_5)_2O$	74.08
Ethyl Acetate	$C_2H_5C_2H_3O_2$	88.06
" Carbamate	$CO(OC_2H_5)NH_2$	89.07
" Chloride	C_2H_5Cl	64.50
" Hydroxide (Ethyl Alcohol)	C_2H_5OH	46.05
Ethylmorphine Hydrochloride	$C_{19}H_{23}O_3NHCl+2H_2O$	385.69
" " Anhydrous	$C_{19}H_{23}O_3NHCl$	349.66
Ethyl Nitrite	$C_2H_5NO_2$	75.05
" Oxide (Ether)	$(C_2H_5)_2O$	74.08
Eucaine (Beta)	$C_{15}H_{21}O_2NHCl+H_2O$	301.66
Eucalyptol (Cineol)	$C_{10}H_{18}O$	154.14
Eugenol	$C_{10}H_{12}O_2$	164.10
Europium	**Eu**	**152.0**
Ferric Acetate	$Fe(C_2H_3O_2)_3$	232.91
" Ammonium Sulphate	$FeNH_4(SO_4)_2+12H_2O$	482.21
" " " Anhydrous	$FeNH_4(SO_4)_2$	266.02
" Chloride	$FeCl_3+6H_2O$	270.32
" " Anhydrous	$FeCl_3$	162.22
" Hydroxide	$Fe(OH)_3$	106.86
" Hypophosphite	$Fe(PH_2O_2)_3$	251.01
" Nitrate	$Fe(NO_3)_3$	241.87
" Oxide	Fe_2O_3	159.68
" Phosphate (normal, not U.S.P.)	$FePO_4$	150.88
" Pyrophosphate (normal, not U.S.P.)	$Fe_4(P_2O_7)_3$	745.60
" Subsulphate (variable)		
" Sulphate (Tersulphate)	$Fe_2(SO_4)_3$	399.89
Ferrous Bromide	$FeBr_2+6H_2O$	323.78
" " Anhydrous	$FeBr_2$	215.68
" Carbonate	$FeCO_3$	115.84
" Iodide	FeI_2	309.68
" Lactate	$Fe(C_3H_5O_3)_2+3H_2O$	287.97
" " Anhydrous	$Fe(C_3H_5O_3)_2$	233.92
" Oxide	FeO	71.84
" Sulphate	$FeSO_4+7H_2O$	278.02
" " Anhydrous	$FeSO_4$	151.91
" " Exsiccated (approximately)	$2FeSO_4+3H_2O$	357.87
" Sulphide	FeS	87.91
Ferrum	**Fe**	**55.84**
Fluorescein (Resorcinolphthalein)	$C_{20}H_{12}O_5$	332.10
Fluorine	**F**	**19.0**

Assay—Dissolve about 1 Gm. of Soluble Ferric Phosphate, accurately weighed, in 25 ml. of water and 5 ml. of hydrochloric acid in a glass-stoppered flask; add 4 Gm. of potassium iodide, securely stopper the flask, and allow the mixture to stand 15 minutes; dilute with 50 ml. of water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch T.S. as the indicator. Perform a blank determination with the same quantities of the reagents and in the same manner and make any necessary correction. Each ml. of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg. of Fe.

Packaging and storage—Preserve Soluble Ferric Phosphate in well-closed, light-resistant containers.

CATEGORY—Hematinic.

USUAL DOSE—**250 mg.** (approximately 4 grains).

Ferric Subsulfate Solution

FERRIC SUBSULFATE SOLUTION

Monsel's Solution — Basic Ferric Sulfate Solution

Ferric Subsulfate Solution is a water solution containing, in each 100 ml., basic ferric sulfate equivalent to not less than 20 Gm. and not more than 22 Gm. of Fe.

Ferrous Sulfate	1045 Gm.
Sulfuric Acid	55 ml.
Nitric Acid,	
Purified Water, each, a sufficient quantity,	
To make	1000 ml.

Add the sulfuric acid to 800 ml. of purified water in a suitable porcelain dish, and heat the mixture nearly to 100°; then add 75 ml. of nitric acid, and mix well. Divide the ferrous sulfate, coarsely powdered, into 4 approximately equal portions, and add these portions one at a time to the hot liquid, stirring after each addition until effervescence ceases. If, after the ferrous sulfate has dissolved, the solution has a black color, add nitric acid, a few drops at a time, with heating and stirring, until red fumes cease to be evolved. Boil the solution until it assumes a red color and is free from nitrate, as indicated by the test below, maintaining the volume at about 1000 ml. by the addition of purified water as needed. Cool, and add enough purified water to make the product measure 1000 ml.; filter, if necessary, until the product is clear.

NOTE: If exposed to low temperatures, crystallization may take place in the Solution. The crystals will redissolve upon warming the Solution.

Description—Ferric Subsulfate Solution is a reddish brown liquid, odorless or nearly so, with a sour, strongly astringent taste. Ferric Subsulfate Solution is acid to litmus, and it is affected by light. Its specific gravity is about 1.548.

A. INGREDIENT NAME:

FERRIC SUBSULFATE SOLUTION

B. Chemical Name:

C. Common Name:

Monsel's Solution, Basic Solution, Iron Hydroxide Sulfate

D. Chemical grade or description of the strength, quality, and purity of the ingredient:

	(Specification)	*(Result)*
Assay	20-22%	21.2%

E. Information about how the ingredient is supplied:

Reddish-Brown liquid, almost odorless, sour, strongly astringent taste, affected by light.

F. Information about recognition of the substance in foreign pharmacopeias:

NFXI

G. Bibliography of available safety and efficacy data including peer reviewed medical literature:

Spitzer, M. and Chernys, A.E. Monsel's solution-induced artifact in the uterine cervix. *Am J Obstet Gynecol,* 1996; 175(5): 1204-1207.

Su, G. B. Clinical experience on efficacy of Monsel's solution. *Chung Hua Wai Ko Tsa Chih,* 1981: 19(11): 685-686.

Manca, D. P. Therapeutic use. Human/ Wound Healing. *Can Fam Physician,* 1997; 43: 1359.

H. Information about dosage forms used:

Solution

I. Information about strength:

20-22mg per 100ml
Undiluted

J. Information about route of administration:

Topically

K. Stability data:

L. Formulations:

Oxidizing ferrous sulfate with nitric acid
See file for compounding directions

M. Miscellaneous Information:

30-1168
54812

CERTIFICATE OF ANALYSIS

PRODUCT: FERRIC SUBSULFATE SOLUTION (PURIFIED) GRADE: --
RELEASE #: 104273 LOT # :B62908M10 CODE:G09-21250/97

	SPECIFICATIONS	RESULT
1. DESCRIPTION	REDDISH-BROWN LIQUID	CONFORMS
2. Identification	To pass test	Passes test
3. Assay (Fe) [gm/100 ml]	20 - 22%	21.2%
4. Nitrate	Negative	Negative
5. Ferrous salts	Negative	Negative
6. Solubility	To pass test	Passes test

ATTENTION: TONY HATCHETT

Date :11/13/97 Prepared by : A. KASHWALA

10907 Approved by : 12/97

Our Order # 239573-1 Your PO # 54504

QUALITY CONTROL REPORT

CHEMICAL NAME.: FERRIC SUBSULFATE (MONSEL'S SOLN)

MANUFACTURE LOT NO.: C63940C26

PHYSICAL TEST

SPECIFICATION TEST STANDARD.: USP__/BP__/MERCK__/NF__/MART.__/CO.SPECS.__.

1) DESCRIPTION.:
REDDISH-BROWN LIQUID; ALMOST ODORLESS; SOUR, STRONGLY ASTRINGENT TASTE; AFFECTED BY LIGHT.

2) SOLUBILITY.:
MISCIBLE WITH WATER AND IN ALCOHOL; ACID TO LITMUS.

3) MELTING POINT.:

4) SPECIFIC GRAVITY.: 1.548.

5) IDENTIFICATION.:
A) FERROUS SALTS TEST GIVES NEGATIVE RESULTS.
B) FERRIC SALTS TEST GIVES POSITIVE RESULTS.

PASSES.: _____ **FAILS.:** _____

COMMENTS.: NOTE - MAY CRYSTALLIZE OR SOLIDIFY AT LOW TEMPERATURES.

ANALYST SIGNATURE.: _____ **DATE.:** _____

PREPACK TEST.: _____ **DATE.:** _____ **INITIAL.:** _____

RETEST.: _____ **DATE.:** _____ **INITIAL.:** _____

MALLINCKRODT BAKER -- FERRIC SUBSULFATE SOLUTION - FERRIC SUBSULFATE SOLUTION
MATERIAL SAFETY DATA SHEET
NSN: 6505012078245
Manufacturer's CAGE: 70829
Part No. Indicator: A
Part Number/Trade Name: FERRIC SUBSULFATE SOLUTION

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General Information

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Item Name: FERRIC SUBSULFATE SOLUTION
Company's Name: MALLINCKRODT BAKER INC.
Company's Street: 222 RED SCHOOL LANE
Company's City: PHILLIPSBURG
Company's State: NJ
Company's Country: US
Company's Zip Code: 08865-2219
Company's Emerg Ph #: 908-859-2151/800-424-9300(CHEMTREC)
Company's Info Ph #: 201-859-2151
Record No. For Safety Entry: 001
Tot Safety Entries This Stk#: 001
Status: SE
Date MSDS Prepared: 22AUG95
Safety Data Review Date: 30OCT96
Supply Item Manager: KX
MSDS Preparer's Name: UNKNOWN
MSDS Serial Number: BNVDB
Specification Number: NONE
Spec Type, Grade, Class: NOT APPLICABLE
Hazard Characteristic Code: J6
Unit Of Issue: BT
Unit Of Issue Container Qty: 500 ML
Type Of Container: STD COML PKG
Net Unit Weight: 3.2 LBS

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Ingredients/Identity Information

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Proprietary: NO
Ingredient: FERRIC SUBSULFATE
Ingredient Sequence Number: 01
Percent: 40-45
NIOSH (RTECS) Number: 1004946FS
CAS Number: 1310-45-8
OSHA PEL: NOT ESTABLISHED
ACGIH TLV: NOT ESTABLISHED
Other Recommended Limit: NONE RECOMMENDED

Proprietary: NO
Ingredient: SULFURIC ACID (SARA III)
Ingredient Sequence Number: 02
Percent:

olution with potassium or sodium hydrate T.S.
itate, without evolving vapor of *ammonia*.
e Solution, diluted with 4 volumes of water, be
g it with an excess of potassium or sodium hydra
htly acidulated with acetic acid, a portion of thi
nd for some time, should not give a white, crystalli
tartrate).
tion of the acidulated and cooled filtrate a little
ed, and the liquid heated to boiling, it should gr
stalline precipitate.
im. of the Solution be introduced into a glass-sto
acity of about 100 Cc.), together with 15 Cc. of wa
ric acid, and, after the addition of 1 Gm. of po
be kept for half an hour at a temperature of 40°
d mixed with a few drops of starch T.S., it should
normal sodium hyposulphite V.S. to discharge th
e liquid (each Cc. of the volumetric solution indic
iron).

itras. Ferri et Ammonii Citras.

RRI ET AMMONII ACETAT

F IRON AND AMMONIUM ACETA

r Ammonii Acetatis, Pharm. 1880. [Basic Mixture.]

ic ride, *twenty cubic centimeters*
cia, *irty cubic centimeters*
ionium Acetate, *two hundred cubic centi*
.....
one hundred cubic centimeters
dred and twenty cubic centimeters
t quantity,

o make *one thousand cubic centimeters*

f Ammonium Acetate (which should not
ly, the Diluted Acetic Acid, the Tinctur
atic Elixir, and the Glycerin, and, last
product measure *one thousand* (1000

should be freshly made, when wanted.

UOR FERRI NITRATIS

JTION OF FERRIC NITRATE.

ion of Ferric Nitrate [$Fe_2(NO_3)_6 = 483.1$
ent. of the anhydrous salt, and corresp
of metallic iron.

Solution of Ferric Sulphate, *one hundred and eighty grammes*	180 Gm.
Ammonia Water, *one hundred and sixty cubic centimeters*	160 Cc.
Nitric Acid, *seventy-one grammes*	71 Gm.
Distilled Water,	
Water, each, *a sufficient quantity,*	
To make *one thousand grammes*	1000 Gm.

Mix the Ammonia Water with *five hundred* (500) *cubic centimeters* of old Water, and the Solution of Ferric Sulphate with *fifteen hundred* (1500) *cubic centimeters* of cold Water. Add the latter solution slowly to the diluted Ammonia Water, with constant stirring. Let the mixture stand until the precipitate has subsided as far as practicable, and then decant the supernatant liquid. Add to the precipitate *one thousand* (1000) *cubic centimeters* of cold Water, mix well, and again set the mixture aside, as before. Repeat the washing with successive portions of cold Water, in the same manner, until the washings produce but a slight cloudiness with barium chloride test-solution. Pour the washed ferric hydrate on a wet muslin strainer, and let it drain thoroughly. Then transfer it to a porcelain capsule, add the Nitric Acid, and stir with a glass rod, until a clear solution is obtained. Finally, add enough Distilled Water to make the finished product weigh *one thousand* (1000) *grammes*. Filter, if necessary.

A clear, amber-colored or reddish liquid, odorless, having an acid, styptic taste, and an acid reaction.
Specific gravity: about 1.050 at 15° C. (59° F.).
The Solution gives a brownish-red precipitate with ammonia water, and a blue one with potassium ferrocyanide T.S.
If a clear crystal of ferrous sulphate be added to a cooled mixture of equal parts of the Solution and of concentrated sulphuric acid, the crystal will become brown and be surrounded by a brownish-black zone.
If 1.12 (1.1176) Gm. of the Solution be introduced into a glass-stoppered bottle (having a capacity of about 100 Cc.), together with 15 Cc. of water and 5 Cc. of hydrochloric acid, and, after the addition of 1 Gm. of potassium iodide, the mixture be kept for half an hour at a temperature of 40° C. (104° F.), then cooled, and mixed with a few drops of starch T.S., it should require about 2.8 Cc. of decinormal sodium hyposulphite V.S. to discharge the blue or greenish color of the liquid (each Cc. of the volumetric solution indicating 0.5 per cent. of metallic iron).

LIQUOR FERRI SUBSULPHATIS.

SOLUTION OF FERRIC SUBSULPHATE.

[Solution of Basic Ferric Sulphate. Monsel's Solution.]

An aqueous solution of Basic Ferric Sulphate (of variable chemical composition), corresponding to about 13.6 per cent. of metallic iron.

Ferrous Sulphate, in clear crystals, *six hundred and seventy-five grammes* 675
Sulphuric Acid, *sixty-five grammes* 65
Nitric Acid,
Distilled Water, each, *a sufficient quantity*,
To make *one thousand grammes* 1000

Add the Sulphuric Acid to *five hundred* (500) *cubic centimetres* Distilled Water in a capacious porcelain capsule, heat the mixture to nearly 100° C. (212° F.), then add *sixty-five* (65) *grammes* of Sulphuric Acid, and mix well. Divide the Ferrous Sulphate, coarsely powdered, into four equal portions, and add these portions, one at a time, to the hot liquid, stirring after each addition until effervescence ceases. When all of the Ferrous Sulphate is dissolved, add a few drops of Nitric Acid, and, if this causes a further evolution of red fumes, continue to add Nitric Acid, a few drops at a time, until it no longer causes red fumes to be evolved; then boil the Solution until it has a ruby-red color and is free from nitrous odor. Lastly, add Distilled Water to make the product weigh *one thousand* (1000) *grammes*. Keep the product in well-stoppered bottles, in a moderately cool place (not under 22° C. or 71.6° F.), protected from light.

This solution will sometimes crystallize, forming a semi-solid mass. When this occurs, the application of a gentle heat to the mass will restore the liquid condition.

NOTE.—Solution of Ferric Subsulphate is to be dispensed. Solution of Persulphate of Iron has been prescribed by the Pharmacopœia.

A dark reddish-brown liquid, odorless or nearly so, of an acid, astyptic taste, and an acid reaction.

Specific gravity: about 1.550 at 15° C. (59° F.).

Miscible with water and alcohol, in all proportions, without decomposition.

The diluted Solution yields a brownish-red precipitate with ammonia, a blue one with potassium ferrocyanide T.S., and a white one, with hydrochloric acid, with barium chloride T.S.

On slowly mixing 2 volumes of the Solution with 1 volume of concentrated sulphuric acid, in a beaker, a semi-solid, white mass will separate out (difference from *tersulphate*).

On adding a clear crystal of ferrous sulphate to a cooled mixture of volumes of concentrated sulphuric acid and a diluted portion of the Solution, the crystal should not become brown, nor should there be a brown color developed around it (absence of *nitric acid*).

If to a small portion of the Solution, diluted with about 10 volumes of water, a few drops of freshly prepared potassium ferricyanide T.S. be added, a pure brown color should be produced, without a tinge of green or blue (absence of *ferrous salt*).

If 1.12 (1.1176) Gm. of the Solution be introduced into a glass-stoppered bottle (having a capacity of about 100 Cc.), together with 15 Cc. of water, 2 Cc. of hydrochloric acid, and, after the addition of 1 Gm. of potassium iodide, the mixture be kept for half an hour at a temperature of

with physiological salt solution and slowly administered intravenously.

Dosage Forms—Injection USP: 50 mg/5 ml, 250 mg/25 ml; for Injection USP: 50 mg.

Other Anticoagulant Antagonists

Tolonium Chloride [Toluidine Blue; Blutene (*Abbott*)] is 3-amino-7-(dimethylamino)-2-methylphenazathionium chloride [$C_{15}H_{16}ClN_3S$]. *Description and Solubility:* A dark-green powder. 1 Gm dissolves in about 26 ml of water, yielding a blue to violet solution, and in about 175 ml of alcohol, yielding a blue solution. *Uses:* It precipitates heparin. It is used for the treatment of overdosage of heparin and for the treatment of certain hemorrhagic states, some of which, but not all, are associated with elevated blood heparinoid levels. The organs are stained blue and the urine becomes pale blue-green. Nausea, vomiting, burning sensation upon urination, and tenesmus may occur, but they may be avoided by adequate fluid intake. *Dose: Oral, 200 to 300 mg daily for menorrhagia; intramuscular or slow intravenous, for heparin overdosage, 100 mg. Veterinary Dose: Oral, Dogs and Cats, 200 to 300 mg daily.*

Hemostatics and Styptics

Many substances not especially related to the clotting mechanism are capable of promoting clotting. Upon contact with most surfaces, platelets disintegrate, thereby liberating a thromboplastin. Spongy and gauzy materials, which provide a large surface area, are thus used to arrest bleeding; absorbable sponges may be left permanently at the site of bleeding. Fibrin, fibrinogen, and thrombin are also potent hemostatics (see page 829). Astringents (see Chapter 43, page 768) also initiate clotting by precipitating proteins and by labilizing platelets; ferric salts are mostly employed as styptics.

Alum—see page 769.

Cellulose, Oxidized—see page 1876.

Estrogens, Conjugated—see page 991.

Ferric Chloride—see page 772.

Fibrinogen—see page 830.

Fibrinogen with Antihemophilic Factor—see page 830.

Absorbable Gelatin Sponge USP

[Gelfoam (*Upjohn*)]

Absorbable Gelatin Sponge is gelatin in the form of a sterile, absorbable, water-insoluble sponge.

Description—A light, nearly white, nonelastic, tough, porous, hydrophilic solid. A 10-mm cube weighing approximately 9 mg will take up approximately 45 times its weight of well-agitated oxalated whole blood. It is stable in dry heat at 150° for 4 hours.

Solubility—Insoluble in water, but absorbable in body fluids; completely digested by a solution of pepsin.

Uses—Absorbable Gelatin Sponge is a *hemostatic* and *coagulant* used to control bleeding. It is moistened with thrombin solution or sterile normal saline and may then be left in place following the closure of a surgical incision. It is absorbed in from 4 to 6 weeks.

Human Antihemophilic Factor—see page 830.

Antihemophilic Human Plasma—see page 830.

Protamine Sulfate—see page 836.

Thrombin—see page 831.

Thromboplastin—see page 1376.

Tolonium Chloride—see this page.

Other Hemostatics and Styptics

Carbazochrome Salicylate [Adrenosem (*Massengill*); Adrestat (*Organon*)]—An adrenochrome monosemicarbazone [3-hydroxy-1-methyl-5,6-indolinedione-5-semicarbazone] sodium salicylate complex [$C_{10}H_{12}N_4O_3 \cdot C_7H_5NaO_3$] occurring as a fine, orange-red, odorless powder with a sweetish saline taste. It is soluble in both alcohol and water. A 13% aqueous solution has a pH range of 6.7–7.3. *Uses:* Proposed for the systemic control of capillary bleeding of various types. Its clinical usefulness for this purpose is scientifically unjustified. *Dose: Oral,* 1 to 5 mg 4 times daily; *intramuscular,* 5 mg every 2 to 4 hours.

Ferric Subsulfate [approx. $Fe_4O(SO_4)_5.H_2O$]—Used and prepared only as a solution. Ferric Subsulfate Solution was official in NF XI. It is prepared by oxidizing ferrous sulfate with nitric acid. The solution contains 20–22 Gm Fe per 100 ml. It is reddish brown and has an astringent, sour taste. It is miscible with alcohol. *Uses:* An important styptic solution. The solution is less irritating than ferric sulfate because of the lesser amount of sulfuric acid present. It is occasionally used to control surface bleeding and as an astringent in a variety of skin disorders. It should not be used in vesicular, bulbous, or exudative dermatoses, because it may then cause permanent pigmentation of the skin.

Fibrin Foam Human—A dry artificial sponge of human fibrin, prepared by clotting with thrombin a foam of a solution of human fibrinogen. The clotted foam is dried from the frozen state and heated at 130° for 3 hours to sterilize. It appears as a fine, white sponge of firm texture. It is insoluble in water. *Uses:* A mechanical coagulant of blood in case of hemorrhage, especially in surgery of the brain, liver, kidneys, and other organs where ordinary methods of hemostasis are ineffective or inadvisable. This preparation is used by impregnating it with a freshly prepared solution of thrombin in normal saline solution and then applying the foam to the bleeding area. In time, the foam is absorbed.

Electrolytes

The concentration of several of the electrolytes in the plasma is critical for the proper functioning of the cells, especially those of the excitable tissues. For the normal plasma concentration of the principal electrolytes, see page 815. The proper balance of the several ions is complex; it depends not alone upon the concentration in the extracellular fluid (of which plasma is one compartment) but also upon the intracellular concentration, the ratio across the cell membrane being an essential factor, and upon the ratio of one ion type to another. Thus, the plasma electrolyte concentrations provide only a crude clue to the electrolyte status of the patient.

Tinc has been exposed to daylight for som
im yields a greenish or bluish color wi
potassium ferricyanide T.S. (*presence of so
ferrous salt*).

Nitrate—Dilute 4 ml. of Ferric Chloride Tin
ure with 10 ml. of water, heat the solution
boiling and pour it into a mixture of 10 ml.
vater and 10 ml. of ammonia T.S. Filter th
nixture while hot, and wash the filter with h
vater until the total filtrate measures 30 m
Mix the filtrate well and to 5 ml. add 2 drops
ndigo carmine T.S. Mix this solution with
nl. of sulfuric acid: the blue color does not d
appear within 1 minute.

Assay—Transfer 5 ml. of Ferric Chloride Tin
ure, accurately measured, to a flask of suitab
capacity. Add about 20 ml. of water, 3 Gm
potassium iodide, and 3 ml. of hydrochloric ac
Allow the solution to stand during 15 minut
dilute it with 100 ml. of water, and then titra
with 0.1 *N* sodium thiosulfate, using starch T
as the indicator. Each ml. of 0.1 *N* sodium thi
sulfate is equivalent to 16.22 mg. of $FeCl_3$.

Alcohol content, page 404—Ferric Chlorid
Tincture contains from 58 to 64 per cent
C_2H_5OH.

Packaging and storage—Preserve Ferric Chl
ride Tincture in tight, light-resistant containe
and avoid exposure to direct sunlight or to exce
sive heat.

CATEGORY—Astringent; hematinic.
USUAL DOSE—**0.6 ml.**

Ferric Citrochloride Tincture

F Citrochloride Tincture is a hydr
alco solution containing, in each 100 m
ferric citrochloride equivalent to not less th
4.48 Gm. of Fe.

Ferric Chloride Solution	350 m
Sodium Citrate	450 G
Alcohol	150 m
Water, a sufficient quantity,	
To make about	1000 m

Mix the ferric chloride solution with 150
of water, dissolve the sodium citrate in
mixture with the aid of gentle heat, and
the alcohol. When the solution has becom
cold, add sufficient water to make the produ
measure 1000 ml. Set the Ferric Citrochlorid
Tincture aside in a cold place for a few day
so that the excess of saline matter may sep
rate, and then filter.

Assay—Transfer 5 ml. of Ferric Citrochlorid
Tincture, accurately measured, into an iodin
flask, add 7 ml. of hydrochloric acid and 25 m
of water, and heat on a water bath until clea
Cool to room temperature and add about 25 m
of water and 3 Gm. of potassium iodide, and all
the mixture to stand for 15 minutes. Then rin
the stopper and the sides of the flask with

additional 50 ml. of water and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch T.S. as the indicator. Each ml. of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg. of Fe.

Alcohol content, page 404—Ferric Citrochloride Tincture contains from 13 to 15 per cent of C_2H_5OH.

Packaging and storage—Preserve Ferric Citrochloride Tincture in tight, light-resistant containers and avoid exposure to direct sunlight or to excessive heat.

CATEGORY—Hematinic.
USUAL DOSE—**0.5 ml.**
One usual dose represents about 22 mg. of iron in the form of ferric citrochloride.

SOLUBLE FERRIC PHOSPHATE

Ferric Phosphate with Sodium Citrate

Soluble Ferric Phosphate is ferric phosphate rendered soluble by the presence of sodium citrate, and yields not less than 12 per cent and not more than 15 per cent of Fe.

Description—Soluble Ferric Phosphate occurs as thin, bright green, transparent scales, or as granules. It is without odor, and has an acid, slightly salty taste. Soluble Ferric Phosphate is stable in dry air when protected from light, but when unprotected, soon becomes discolored. A solution of Soluble Ferric Phosphate (1 in 10) is acid to litmus.

Solubility—Soluble Ferric Phosphate dissolves freely in water. It is insoluble in alcohol.

Identification—

A: To 10 ml. of a solution of Soluble Ferric Phosphate (1 in 100) add ammonia T.S., dropwise: the solution becomes reddish brown, but no precipitate forms.

B: Remove the iron from 10 ml. of a solution of Soluble Ferric Phosphate (1 in 10) by boiling it with an excess of sodium hydroxide T.S.; filter, and strongly acidify the filtrate with hydrochloric acid: a cooled portion of this liquid mixed with an equal volume of magnesia mixture T.S. and treated with a slight excess of ammonia T.S. produces an abundant, white, crystalline precipitate. This precipitate, after being washed, turns greenish yellow when treated with a few drops of silver nitrate T.S. (*distinction from pyrophosphate*).

Ammonium salts—Boil about 100 mg. of Soluble Ferric Phosphate with 5 ml. of sodium hydroxide T.S.: a reddish brown precipitate forms without the evolution of ammonia.

Lead—Dissolve 1 Gm. of Soluble Ferric Phosphate in 3 ml. of nitric acid (1 in 2) in a 100-ml. volumetric flask. Add sufficient water to make 100 ml., and mix well. A 10-ml. portion of this solution contains no more than 5 mcg. of lead (corresponding to not more than 50 parts per million) when treated according to the *Lead Limit Test*, page 414, using 10 ml. of ammonium citrate solution, 3 ml. of potassium cyanide solution, and 1 ml. of hydroxylamine hydrochloride solution.

Assay—Dissolve about 1 Gm. of Soluble Ferric Phosphate, accurately weighed, in 25 ml. of water and 5 ml. of hydrochloric acid in a glass-stoppered flask; add 4 Gm. of potassium iodide, securely stopper the flask, and allow the mixture to stand 15 minutes; dilute with 50 ml. of water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch T.S. as the indicator. Perform a blank determination with the same quantities of the same reagents and in the same manner and make any necessary correction. Each ml. of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg. of Fe.

Packaging and storage—Preserve Soluble Ferric Phosphate in well-closed, light-resistant containers.

CATEGORY—Hematinic.
USUAL DOSE—**250 mg.**

FERRIC SUBSULFATE SOLUTION

Monsel's Solution; Basic Ferric Sulfate Solution

Ferric Subsulfate Solution is a water solution containing, in each 100 ml., basic ferric sulfate equivalent to not less than 20 Gm. and not more than 22 Gm. of Fe.

NOTE: If exposed to low temperatures, crystallization may take place in the Solution. The crystals will redissolve upon warming the Solution.

Description—Ferric Subsulfate Solution is a reddish brown liquid, odorless or nearly so, with a sour, strongly astringent taste. Ferric Subsulfate Solution is acid to litmus, and it is affected by light. Its specific gravity is about 1.548.

Solubility—Ferric Subsulfate Solution is miscible with water and with alcohol.

Identification—Separate portions of a dilution of Ferric Subsulfate Solution (1 in 20) yield a brownish red precipitate with ammonia T.S., a blue precipitate with potassium ferrocyanide T.S., and a white precipitate, insoluble in hydrochloric acid, with barium chloride T.S.

Nitrate—Add a clear crystal of ferrous sulfate to a cooled mixture of equal volumes of sulfuric acid and a dilution of Ferric Subsulfate Solution (1 in 10): the crystal does not become brown, nor does a brownish black color develop around it.

Ferrous salts—Add a few drops of freshly prepared potassium ferricyanide T.S. to 2 ml. of a dilution of Ferric Subsulfate Solution (1 in 20): a brown color is produced and the solution remains free from even a transient green or greenish blue color.

Assay—Dilute about 10 ml. of Ferric Subsulfate Solution, accurately measured, to exactly 100 ml. with water. Transfer 10 ml. of the dilution to a stoppered flask; add 5 ml. of hydrochloric acid and 3 Gm. of potassium iodide.

Stopper the flask, and allow the mixture to stand for 15 minutes; then dilute with 50 ml. of water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch T.S. as the indicator. Each ml. of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg. of Fe.

Packaging and storage—Preserve Ferric Subsulfate Solution in tight, light-resistant containers, and in a moderately warm place (not under 22°).

CATEGORY—Astringent.

AS A STYPTIC—Use Ferric Subsulfate Solution undiluted.

FERROUS CARBONATE PILLS

Chalybeate Pills
Blaud's Pills
Ferruginous Pills

Each Pill contains not less than 60 mg. of $FeCO_3$.

Ferrous Sulfate, in clear crystals	16 Gm.
Potassium Carbonate	9.5 Gm.
Sucrose, finely powdered	4 Gm.
Tragacanth, finely powdered	1 Gm.
Althea, in very fine powder	1 Gm.
Glycerin,	
Purified Water, each, a sufficient quantity,	
To make 100 pills.	

Triturate the potassium carbonate in a mortar with a sufficient quantity (about 5 drops) of glycerin, add the ferrous sulfate and sucrose, previously triturated together to a uniform, fine powder, and mix the mass thoroughly until it assumes a greenish color. When the reaction is complete, incorporate the tragacanth and althea, and add purified water, if necessary, to obtain a mass of pilular consistency. Divide it into 100 pills.

Assay—Carefully pulverize 5 Ferrous Carbonate Pills in a mortar, and triturate with 20 ml. of diluted sulfuric acid until all carbonate is dissolved. Transfer completely the contents of the mortar to a beaker of about 800-ml. capacity, and add water to bring the total volume to approximately 300 ml. Add orthophenanthroline T.S., and titrate immediately with 0.1 *N* ceric sulfate, avoiding excessive stirring. Near the end of the titration tilt the beaker at an angle of 45° to facilitate the detection of the end point. Each ml. of 0.1 *N* ceric sulfate is equivalent to 11.59 mg. of $FeCO_3$.

Packaging and storage—Preserve Ferrous Carbonate Pills in well-closed containers.

CATEGORY—Hematinic.

USUAL DOSE—5 pills.

FERROUS IODIDE SYRUP

Ferrous Iodide Syrup contains, in each 100 ml., not less than 6.5 Gm. and not more than 7.5 Gm. of FeI_2, representing approximately 5 per cent of FeI_2, by weight.

Ferrous Iodide Syrup may be prepared as follows:

Iron, in the form of fine, bright wire	20 Gm.
Iodine	60 Gm.
Hypophosphorous Acid	5 ml.
Sucrose	850 Gm.
Purified Water, a sufficient quantity,	
To make	1000 ml.

NOTE: For the purpose of retarding discoloration, 1.3 Gm. of citric acid may replace the hypophosphorous acid in the above formula.

Place the iron in a flask having a capacity of about 500 ml., add the iodine and 200 ml. of purified water, and shake the mixture occasionally, checking the reaction, if necessary, by placing the flask in cold water. When the liquid has acquired a green color and has lost the odor of iodine, heat it to boiling, and dissolve 100 Gm. of sucrose in the hot liquid. Filter the solution at once into a flask graduated to 1000 ml. and containing the remainder of the sucrose, and rinse the flask containing the iron with 240 ml. of hot purified water in divided portions, passing the rinsings successively through the filter. Agitate the mixture until the sucrose is dissolved, warming if necessary, cool to 25°, and add the hypophosphorous acid and enough purified water to make the product measure 1000 ml. Mix, and strain.

Description—Ferrous Iodide Syrup is a transparent, pale, yellowish green, syrupy liquid having a sweet, ferruginous taste and a slight acid reaction. Its specific gravity is about 1.

Identification—

A: Add a few drops of potassium ferricyanide T.S. to 5 ml. of Ferrous Iodide Syrup: a blue precipitate forms.

B: Mix 5 ml. of Ferrous Iodide Syrup with a few drops of starch T.S., and add 3 drops of chlorine T.S.: the liquid acquires a deep blue color.

Free iodine—To about 5 ml. of Ferrous Iodide Syrup add a few drops of starch T.S.: no blue color is produced.

Assay—Place exactly 10 ml. of Ferrous Iodide Syrup in a flask, dilute it with 30 ml. of water, add 50.0 ml. of 0.1 *N* silver nitrate, and 5 ml. of nitric acid, and heat on a water bath until the precipitate of silver iodide is greenish yellow. Cool, add 2 ml. of ferric ammonium sulfate T.S., and determine the residual silver nitrate by titra-

...ied Ferrous Sulphate (5064-n)

...ed Ferrous Sulfate (USAN); Exsiccated Ferrous Sulphate; ...rosi Sulfas Exsiccatus.

CAS — 13463-43-9.

...rmacopoeias. In Aust., Br., Int., and US.

...errous sulphate deprived of part of its water of crystallisation by drying at 40°. The USP specifies that it consists primarily of the monohydrate with varying amounts of the tetrahydrate. A greyish-white to buff-coloured powder. The BP specifies 86 to 90% of $FeSO_4$; the USP specifies 86 to 89% of $FeSO_4$.

...lowly but almost completely **soluble** in freshly boiled and cooled water; practically insoluble in alcohol.

...Ferrous sulphate is employed for iron-deficiency anaemia. It is given by mouth and the dried form is frequently used in solid dosage forms and the heptahydrate in liquid dosage forms. Usual doses of dried ferrous sulphate are up to 600 mg daily (equivalent to 180 to 195 mg of iron daily, this figure being somewhat variable depending on the purity and water content of the salt).

For discussion of iron-deficiency anaemia and its treatment, see p.747; for further discussion of iron and its dosage, see p.1368.

Ferrous sulphate oxidised with nitric and sulphuric acids yields ferric subsulphate solution, also known as Monsel's solution, which has been used as a haemostatic.

Preparations

Names of preparations are listed below; details are given in Part 3.

Official Preparations

BP 1993: Ferrous Sulphate Tablets; Paediatric Ferrous Sulphate Oral Solution;

BPC 1973: Compound Ferrous Sulphate Tablets;

USP 23: Ferrous Sulfate Oral Solution; Ferrous Sulfate Syrup; Ferrous Sulfate Tablets.

Proprietary Preparations

Aust.: Ferrograd C; Ferro-Gradumet; Infa-Tardyferon; Tardyferon; ***Austral.:*** Feritard†; Ferro-Gradumet; Fespant†; Slow-Fe†; ***Belg.:*** Fer-In-Sol; Ferograd; Fero-Gradumet; Resoferon; ***Canad.:*** Fer-In-Sol; Fero-Grad; Fero-Grad-500; Novo-Ferrosulfa; Slow-Fe; ***Eire:*** Feospan; Fer-In-Sol; Ferrograd; Ferrograd C; Slow-Fe; ***Ger.:*** Ce-Ferro forte†; Dreisafer; Eisen-Diasporal; Eisendragees-tiopharm†; Eryfer; Ferro 66 DL†; Hämatopan; Haemoprotect; Kendural C; Plastufer; Resoferix; Tardyferon; Taxofit Mineral Eisen†; Vitaferro; ***Ital.:*** Eryfer; Ferro-Grad; Ferro-Grad C; ***Neth.:*** Eryfer†; Fero-Gradumet; Liquifer; Plexafer†; Resoferon†; ***Norw.:*** Duroferon; Ferromax; Ferro-Retard; ***S.Afr.:*** Fero-Grad; Fesofor; ***Spain:*** Fero-Gradumet; ***Swed.:*** Duroferon; ***Switz.:*** Ferro-Gradumet; Résoféron; ***UK:*** Feospan; Ferrograd; Ferrograd C; Ironorm; Slow-Fe; ***USA:*** Feosol; Feratab; Fer-gen-sol; Fer-In-Sol; Fer-Iron; Fero-Grad-500; Fero-Gradumet; Ferospace; Ferralyn Lanacaps; Ferra-TD; Irospan; Mol-Iron; Slow-Fe.

Multi-ingredient preparations. ***Aust.:*** Aktiferrin; Aktiferrin compositum; Ferrograd-Fol; Kephalodoron; Tardyferon-Fol; ***Austral.:*** Fefol; Feritard-Folic†; FGF Tabs; ***Canad.:*** Fero-Folic†; Iberet; Slow-Fe Folic; ***Eire:*** Fefol; Fefol-Vit; Ferrograd Folic; Fespvit; Folferite; Pregnavite Forte F; Slow-Fe Folic; ***Fr.:*** Fero-Grad vitaminé C; Fero-Grad-500†; Ionarthrol; Pilules Pink†; Tardyferon; Tardyferon B_9; ***Ger.:*** Aktiferrin; Aktiferrin E F; Eryfer comp.; Ferro Cytofol†; ferro sanol comp; Ferro-Folgamma; Ferro-Folsäure-Vicotrat†; Ferro-Folsan; Ferro-Folsan plus†; Ferrophor†; Hämatopan F; Kendural-Fol-500; Kendural-Plus; Plastulen N; Tardyferon-Fol; ***Ital.:*** Cura; Ferro-Grad Folic; Vitamucin con Ferro†; ***Norw.:*** Pregnifer; ***S.Afr.:*** Effee; Fefol; Fefolvit; Fero-Folic; Foliglobin; Iberet; Laxicaps; ***Spain:*** Ferriwas B12 Fuerte; Ferrace; Iberet; Pildoras Ferrug Sanatori; Tardyferon; ***Switz.:*** Actiferrine; Actiferrine-F; Fero-Folic-500; gyno-Tardyferon; infa-Tardyferon†; Kendural; Résoféron fol B†; Tardyferon; ***UK:*** Bidor; Dencyl; Ditemic; Fefol; Fefol Z; Fefol-Vit; Feospan Z†; Feravol†; Ferrograd Folic; Fesovit Z; Fesovit†; Folicin†; Fortespan; Irofol C†; Ironorm; Ironplan†; Pregnavite Forte F; Slow-Fe Folic; ***USA:*** Aqua Ban Plus; Fero-Folic-500; Generet; Gerivites; Iberet; Iberet-Folic-500; Multibret Hematinic; Multibret-Folic; Reticulex†.

Ferrous Tartrate (5065-h)

Ferrosi Tartras.

$C_4H_4FeO_6.2\frac{1}{2}H_2O = 249.0$.

CAS — 2944-65-2 *(anhydrous ferrous tartrate).*

Ferrous tartrate is employed for iron-deficiency anaemia. It is given by mouth in doses of up to 1 g daily (equivalent to up to 224 mg of iron daily).

For a discussion of iron-deficiency anaemia and its treatment, see p.747; for further discussion of iron and its dosage, see p.1368.

† The symbol † denotes a preparation no longer actively ...

Folic Acid (7860-f)

Folic acid is a member of the vitamin B group which is essential for DNA synthesis and some amino-acid conversions and is involved in formate metabolism. Deficiency may result in megaloblastic anaemia. It is given by mouth for folate deficiency states, and also has a role in the prevention of neural tube defects. It should not be given for undiagnosed megaloblastic anaemia that may be due to vitamin B_{12} deficiency, since it may mask continuing neurological degeneration.

Folic Acid *(BAN, rINN).*

Acidum Folicum; Folacin; Folinsyre; PGA; Pteroylglutamic Acid; Pteroylmonoglutamic Acid. *N*-[4-(2-Amino-4-hydroxypteridin-6-ylmethylamino)benzoyl]-L(+)-glutamic acid.

$C_{19}H_{19}N_7O_6 = 441.4$.

CAS — 59-30-3 *(folic acid);* 6484-89-5 *(sodium folate).*

Pharmacopoeias. In *Aust., Belg., Br., Chin., Cz., Eur., Fr., Ger., Hung., Int., It., Jpn, Neth., Port., Swiss,* and *US.*

The standards of *Ph. Eur.* apply to those countries that are parties to the Convention on the Elaboration of a European Pharmacopoeia, see p.xiii.

A yellow to orange brown, odourless or almost odourless crystalline powder.

BP solubilities are: practically insoluble in water and most organic solvents. USP solubilities are: very slightly soluble in water; insoluble in alcohol, acetone, chloroform, and ether. It readily dissolves in dilute solutions of alkali hydroxides and carbonates; soluble in hydrochloric acid and sulphuric acid. The USP injection has a pH of 8 to 11. **Protect** from light.

Stability in solution. A review of the compatibility and stability of components of total parenteral nutrition solutions when mixed in 1- or 3-litre flexible containers.[1] Folic acid has been reported to precipitate in some proprietary amino acid solutions and in the presence of high concentrations of calcium ions, but it appears to be stable and remain in solution provided the pH remains above 5. There have also been reports of folic acid being absorbed by the polyvinyl chloride container and administration set; however other studies have not substantiated such observations.

1. Allwood MC. Compatibility and stability of TPN mixtures in big bags. *J Clin Hosp Pharm* 1984; **9**: 181–98.

Adverse Effects

Folic acid is generally well tolerated. Gastro-intestinal disturbances may occur. Hypersensitivity reactions have been reported rarely.

Precautions

Folic acid should never be given alone or in conjunction with inadequate amounts of vitamin B_{12} for the treatment of undiagnosed megaloblastic anaemia, since folic acid may produce a haematopoietic response in patients with a megaloblastic anaemia due to vitamin B_{12} deficiency without preventing aggravation of neurological symptoms. This masking of the true deficiency state can lead to serious neurological damage, such as subacute combined degeneration of the cord.

Caution is advised in patients who may have folate-dependent tumours.

Interactions. Folate status may be affected by a number of drugs and anticonvulsants, oral contraceptives, antituberculous drugs, alcohol, and folic acid antagonists including aminopterin, methotrexate, pyrimethamine, trimethoprim, and sulphonamides have all been said to produce folate deficiency states.[1] The authors of this review discuss possible mechanisms responsible for the folate deficiency and assess the clinical significance concluding that in some instances, such as during methotrexate or anticonvulsant therapy, replacement therapy with folic acid may become necessary in order to prevent megaloblastic anaemia developing.

Anticonvulsant-associated folate deficiency is discussed further under phenytoin, p.381.

1. Lambie DG, Johnson RH. Drugs and folate metabolism. *Drugs* 1985; **30**: 145–55.

Pharmacokinetics

Folic acid is rapidly absorbed from normal diets, mainly from the proximal part of the small intestine, and is distributed in body tissues. The principal storage site is the liver; it is also actively concentrated in the cerebrospinal fluid. Dietary folates are stated to be less well absorbed than crystalline folic acid. The naturally occurring folate polyglutamates are largely deconjugated and reduced prior to absorption but once absorbed, folic acid is reconverted via dihydrofolate to tetrahydrofolate and conjugated within the cells to form active polyglutamates. It is the 5-methyltetrahydrofolate which appears in the portal circulation, where it is extensively bound to plasma proteins.

There is an enterohepatic circulation for folate; about 4 to 5 µg is excreted in the urine daily. Administration of larger doses of folic acid leads to proportionately more of the vitamin being excreted in the urine. Folate is distributed into breast milk.

Human Requirements

Body stores of folate in healthy persons have been reported as being between 5 to 10 mg, but may be much higher. About 150 to 200 µg of folate a day is considered a suitable average intake for all healthy persons except women of child-bearing potential and pregnant women who require additional folic acid to protect against neural tube defects in their offspring (see below). Folate is present, chiefly combined with several L(+)-glutamic acid moieties, in many foods, particularly liver, kidney, yeast, nuts, and leafy green vegetables. The vitamin is readily oxidised to unavailable forms and is easily destroyed during cooking.

In the United Kingdom dietary reference values (see p.1352) have been published for folate[1] and in the United States recommended daily allowances have been set.[2] Differing amounts are recommended for infants and children of varying ages, for adult males and females, and for pregnant and lactating women. The special folate requirements to protect against neural tube defects are discussed below. In the UK the reference nutrient intake (RNI) for adult males and females is 200 µg daily and the estimated average requirement (EAR) is 150 µg daily. In the USA the allowances published have also recognised that diets containing lower amounts of folate maintain an adequate status and thus the allowance has been set at 200 µg for adult males and 180 µg for adult females.

Folate requirements are increased during pregnancy, apparently due to increased metabolic breakdown of folate rather than foetal transfer;[3] an RNI of 300 µg daily has been suggested for pregnant women in the UK and an RDA of 400 µg daily in the USA. However, McPartlin and colleagues have suggested from a study in 6 pregnant women that these figures are too low, and that intakes of about 450 to 650 µg daily might be more appropriate.[3] Interestingly, in the light of recent confirmation of the value of folate in preventing neural tube defects, it is now recommended that women planning a pregnancy receive a total daily intake of about 600 µg daily, before conception and during the first trimester, which would go some way towards supplying this increased amount.

A number of authorities in the UK and USA have advocated folic acid supplementation of bread or flour to increase the intake in women of childbearing age.[4-7] However, there remains some debate over the appropriateness of such action, and the risks of masking underlying vitamin B_{12} deficiency.[8,9]

1. DoH. Dietary reference values for food energy and nutrients for the United Kingdom: report of the panel on dietary reference values of the committee on medical aspects of food policy. *Report on health and social subjects 41*. London: HMSO, 1991.
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5. Committee on Genetics of the American Academy of Pediatrics. Folic acid for the prevention of neural tube defects. *Pediatrics* 1993; **92**: 493–4.
6. Schorah CJ, Wild J. Fortified foods and folate intake in women of child-bearing age. *Lancet* 1993; **341**: 1417.
7. Sutcliffe M, *et al.* Prevention of neural tube defects. *Lancet* 1994; **344**: 1578.
8. Horton R. Fighting about folate. *Lancet* 1994; **344**: 1696.
9. Wald NJ, Bower C. Folic acid, pernicious anaemia, and prevention of neural tube defects. *Lancet* 1994; **343**: 307.

Uses and Administration

Folic acid is a member of the vitamin B group. Foli acid is reduced in the body to tetrahydrofolate which is a coenzyme for various metabolic processes including the synthesis of purine and pyrimidin nucleotides, and hence in the synthesis of DNA; it i also involved in some amino-acid conversions, an

TITLE:	**Monsel's solution-induced artifact in the uterine cervix.**
AUTHOR:	**Spitzer M; Chernys AE**
AUTHOR AFFILIATION:	**Department of Obstetrics and Gynecology, Queens Hospital Center, Jamaica, NY 11432, USA.**
SOURCE:	**Am J Obstet Gynecol 1996 Nov;175(5):1204-7**
NLM CIT. ID:	**97097948**
ABSTRACT:	**We documented and quantified Monsel's solution-related artifacts after cervical biopsies. All loop electrosurgical cone biopsy specimens over a 3-month period were reviewed for necrosis artifact of the surface epithelium. The degree of change was quantified and correlated with the antecedent use of Monsel's solution. Twenty-four cone biopsy specimens were evaluated. Three of the eight cone biopsy specimens obtained fewer than 10 days after the use of Monsel's solution showed definite changes. Between 10 and 18 days after the use of Monsel's solution, four of eight specimens showed change. After 18 days, none of eight specimens showed change. One specimen at 18 days showed focal changes that seemed to be related to the use of an unusually large amount of Monsel's solution, because the patient had had six biopsies within 2 days. The routine use of Monsel's solution may interfere with the ability to recognize and characterize disease process in cone biopsy specimens when the cone procedure is done within 3 weeks after the use of Monsel's solution.**
MAIN MESH SUBJECTS:	**Cervix Uteri/DRUG EFFECTS/*PATHOLOGY** **Ferric Compounds/*ADVERSE EFFECTS** **Sulfates/*ADVERSE EFFECTS**
ADDITIONAL MESH SUBJECTS:	**Artifacts** **Biopsy** **Female** **Human**
PUBLICATION TYPES:	**JOURNAL ARTICLE**
LANGUAGE:	**Eng**
REGISTRY NUMBERS:	**0 (Ferric Compounds)** **0 (Sulfates)** **1310-45-8 (ferric subsulfate solution)**

Order Documents | 92 71 67 Other Years | Log off IGM
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National Library of Medicine: IGM Full Record Screen

TITLE:	**Stopping cervical bleeding.**
AUTHOR:	**Manca DP**
SOURCE:	**Can Fam Physician 1997 Dec;43:2121**
NLM CIT. ID:	**98088367**
MAIN MESH SUBJECTS:	***Cervix Uteri/INJURIES** **Ferric Compounds/*ADMINISTRATION & DOSAGE** **Hemorrhage/ETIOLOGY/*THERAPY** **Hemostatics/*ADMINISTRATION & DOSAGE** **Sulfates/*ADMINISTRATION & DOSAGE**
ADDITIONAL MESH SUBJECTS:	**Biopsy/ADVERSE EFFECTS** **Female** **Human** **Time Factors**
PUBLICATION TYPES:	**JOURNAL ARTICLE**
LANGUAGE:	**Eng**
REGISTRY NUMBERS:	**0 (Ferric Compounds)** **0 (Hemostatics)** **0 (Sulfates)** **1310-45-8 (ferric subsulfate solution)**

Order Documents | 92 67 71 Other Years | Log off IGM
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National Library of Medicine: IGM Full Record Screen

TITLE:	**[Clinical experience on efficacy of Monsel's solution (author's transl)]**
AUTHOR:	**Su GB**
SOURCE:	**Chung Hua Wai Ko Tsa Chih 1981 Nov;19(11):685-6**
NLM CIT. ID:	**82185983**
MAIN MESH SUBJECTS:	**Ferric Compounds/*THERAPEUTIC USE** **Hemorrhage/*DRUG THERAPY** **Hemostatics/*THERAPEUTIC USE** **Iron/*THERAPEUTIC USE** **Sulfates/*THERAPEUTIC USE**
ADDITIONAL MESH SUBJECTS:	**Adult** **Aged** **Case Report** **English Abstract** **Human** **Male**
PUBLICATION TYPES:	**JOURNAL ARTICLE**
LANGUAGE:	**Chi**
REGISTRY NUMBERS:	**0 (Ferric Compounds)** **0 (Hemostatics)** **0 (Sulfates)** **1310-45-8 (ferric subsulfate solution)** **7439-89-6 (Iron)**

National Library of Medicine: IGM Full Record Screen

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TITLE:	**Practice tips. Mole no more.**
AUTHOR:	**Manca DP**
SOURCE:	**Can Fam Physician 1997 Aug;43:1359**
NLM CIT. ID:	**97411097**
MAIN MESH SUBJECTS:	**Ferric Compounds/*THERAPEUTIC USE** **Hemostatics/*THERAPEUTIC USE** **Nevus/*SURGERY** **Skin Neoplasms/*SURGERY** **Sulfates/*THERAPEUTIC USE**
ADDITIONAL MESH SUBJECTS:	**Human** **Wound Healing**
PUBLICATION TYPES:	**JOURNAL ARTICLE**
LANGUAGE:	**Eng**
REGISTRY NUMBERS:	**0 (Ferric Compounds)** **0 (Hemostatics)** **0 (Sulfates)** **1310-45-8 (ferric subsulfate solution)**

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Practice Tips

Donna P. Manca, MD, CCFP

Stopping cervical bleeding

Indications

This technique can be used for controlling cervical bleeding from a biopsied site. Occasionally, women present to me with bleeding after cervical biopsy or laser treatment for abnormal Pap smears. When they do present, bleeding is usually minimal and settles with watchful waiting. Occasionally a low-grade infection requires antibiotic treatment. On two occasions in my practice, bleeding was excessive and persistent after cervical biopsy or laser or loop excision. Examination of the cervix revealed an oozing injured site. I was able to stop the bleeding by applying Monsel's solution (20% ferric subsulfate) to the cervix.

This method is contraindicated when bleeding is from inside the os cervix or when excessive hemorrhaging requires further intervention.

Procedure

With ringed forceps and gauze, dab the cervix to identify the site of bleeding. Then, using ringed forceps with 2×2 gauze soaked in Monsel's solution, apply the solution directly to the bleeding site.

Discussion

The women I treated were discharged with no further complications or complaints.

Upon reviewing the literature, I found that Monsel's solution is often used in gynecologic oncology for bleeding from cervical and vaginal biopsies. One report[1] described a brownish vaginal discharge that appeared several days after the application of Monsel's when a vaginal pack soaked in Monsel's had been used. Monsel's solution is recommended over sutures after cold-knife conization in studies comparing these two methods of hemostasis.[2,3] Monsel's solution also has been used in examining the vagina for papilloma virus and neoplasia.[4]

I could find no information in the literature on adverse effects for future Pap smears. Necrosis artifact could appear if cervical biopsy is performed within 3 weeks after application of Monsel's solution.[5] Monsel's solution appears to be a safe method for controlling cervical bleeding in this situation. Be aware that biopsies done within 3 weeks of using Monsel's solution can show artifacts. ❖

References

1. Rarliff C. Preventing cervical bleeding with Monsel's solution. *Oncol Nurs Forum* 1992;19(4):664.
2. Gilbert L, Saunders NJ, Stringer R, Sharp F. Hemostasis and cold knife cone biopsy: a prospective, randomized trial comparing a suture versus non-suture technique. *Obstet Gynecol* 1989; 74(4):640-3.
3. Tangtrakul S, Srisupundit S, Linasmita V, Bullangpoti S, Israngura N, Wilailak S, et al. A randomized study comparing suture with non-suture cold-knife conization. *J Obstet Gynecol* 1995;21(6):587-91.
4. Davis GD. Colposcopic examination of the vagina. *Obstet Gynecol Clin North Am* 1993;20(1):217-29.
5. Spitzer M, Chernys AE. Monsel's solution–induced artifact in the uterine cervix. *Am J Obstet Gynecol* 1996;175(5):1204-7.

...

We encourage readers to share some of their practice experience: the neat little tricks that solve difficult clinical situations. *Canadian Family Physician* will pay $50 to authors upon publication of their practice tips.

Dr Manca, *a Fellow of the College, practises family medicine in Edmonton.*

A. INGREDIENT NAME:

DIMERCAPTO-1-PROPANESULFONIC (DMPS)

B. Chemical Name:

DL-2, 3-Dimercapto-1-Propanesulfonic

C. Common Name:

DMPS, Unithiol, Dimaval, Mercuval

D. Chemical grade or description of the strength, quality, and purity of the ingredient:

	(Limit-Min/Max)	*(Results)*
Assay: (Iodometric)	95%	98.2%

E. Information about how the ingredient is supplied:

Fine, white, crystalline powder, odorless

F. Information about recognition of the substance in foreign pharmacopeias:

G. Bibliography of available safety and efficacy data including peer reviewed medical literature:

Aposhian, H.V. DMSA and DMPS– water soluble antidotes for heavy metal poisoning. *Annual Review of Pharmacology and Toxicology,* 1983; 23: 193-215.

Aposhian, H. V., Maiorino, R. M., and Gonzalez-Ramirez, D. Mobilization of heavy metals by newer,therapeutically useful chelating agents. *Toxicology.* 1995; 97(1-3): 23-28.

Chisolm, J. J. BAL, EDTA, DMSA, and DMPS in the treatment of lead poisoning in children. *Clinical Toxicology,* 1992; 30(4): 493-504.

Torrs-Alanis, O., Garza-Ocanas, L., and Pineyro-Lopez, A. Evaluation of Urinary Mercury Excretion After Administration of 2,3-Dimercapto-1-propane Sulfonic Acid to Occupationally Exposed Men. *Cinical Toxicology,* 1995; 33(6): 717-720.

Aposhian, H. V., Maiorino, R. M., and Rivera, , M. Human Studies with the Chelating Agents, DMPS and DMSA. *Clinical Toxicology, 1992; 30(4): 505-528.*

Clarkson, T. W., magos, L., and Cox, C. Tests of Efficacy of Antidotes for Removl of Methylmercury in Human Poisoning during the Iraq Outbreak. *The Journal of Pharmacology and Experimental Therapeutics.* 1981; 218: 74-83.

Reynolds, J. E. *Martindale - The extra Pharmacopeia* (31st ed.). London, UK: the Royal Pharmaceutical Society. 1996. 997.

Chisolm, J. J. and Thomas D. J. Use of 2,3-dimercaptopropane-1-Sulfonate in Treatment of Lead Poisoning inChildren. *The Journal of pharmacology and Experimental therapeutics,* 1985; 235(3): 665-669.

Maiorino, R. M., Gonzalez-Ramirez, D., and Zuniga-Charles, M. Sodium 2, 3-Dimercaptopropane-1-Sulfonate Challenge Test for Mercury in Humans. III. Urinary Mercury after Exposure to Mercurous Chloride. *The Journal of Pharmacology and Experimental therapeutics,* 1996; 277(2): 938-944.

Moore, D. F., O'Callaghan, C. A., and Berlyne, G. Acute arsenic poisoning: absence of polyneuropathy after treatment with 2, 3-dimercaptopropanesulphonate (DMPS). *Journal of Neurology, Neurosurgery, and Psychiatry,* 1994; 57: 1133-1135.

Hurlbut, K. M., Maiorino, R. M., and Mayersohn, M. Determination and Metabolism of Dithiol Chelating Agents XVI: Pharmacokinetics of 2,3-Dimercapto-1-Propanesulfonate ater Intravenous Administration to Human Volunteers. *The Journal of Pharmacology and Experimental Therapeutics.* 1994; 268(2): 662-668.

Maiorino, R. M., Xu, Z., and Aposhian, H. V. Determination and Metaboliosm of Dithiol Chelating Agents. XVII. In Humans, Sodium 2,3-Dimercapto-1-Propanesulfonate is Bound to Plasma Albumin Via Mixed Disulfide Formation and is Found in the Urine as Cyclic Polymeric Disulfides. *The Journal of Pharmacology and Experimental Therapeutics,* 1996: 277(1): 375-384.

Aposhian, H. V., Mershon, M. M., and Brinkley, F. B. Anti-lewisite activity anx stability of meso-dimercaptosuccinic acid and 2,3-dimercapto-1-propanesulfonic acid. *Life Sciences,* 1982; 31(19): 2149-2156.

Aposhian, H. V. Biological Chelation: 2,3-dimercapto-propanesulfonic acid and meso-deimercaptosuccinic acid. *Adv Enzyme Regul,* 1982;20: 301-319.

H. Information about dosage forms used:

Capsules

I. Information about strength:

200-400mg

J. Information about route of administration:

Orally

K. Stability data:

Melts at about 230-235°
Stable

L. Formulations:

M. Miscellaneous Information:

CERTIFICATE OF ANALYSIS

30-2205
IF 5627

Date: 02/02/98

PRODUCT: DL-2,3-DIMERCAPTO-1-PROPANESULFONIC ACID SOD -

CATALOG NO: YY110 **
LOT NO: NA0487

CUSTOMER NO: PRO055

DESCRIPTION	LIMIT MIN.	LIMIT MAX.	RESULT
ASSAY (IODOMETRIC)	95 %	–	98.2 % D

APPROVED BY: Lilian D. Casabar
LILIAN D. CASABAR

2/98

QUALITY CONTROL REPORT

CHEMICAL NAME.: DIMERCAPTO-1-PROPANESULFONIC

MANUFACTURE LOT NO.: NA0487

PHYSICAL TEST

SPECIFICATION TEST STANDARD.: USP__/BP__/MERCK__/NF__/MART.__/CO.SPECS.__.

1) DESCRIPTION.:
FINE, WHITE, CRYSTALLINE POWDER; ODORLESS.

2) SOLUBILITY.:
FREELY SOLUBLE IN WATER; SLIGHTLY SOLUBLE IN ALCOHOL AND IN METHANOL.

3) MELTING POINT.:
MELTS AT ABOUT 230-235 DEGREE.

4) SPECIFIC GRAVITY.:

5) IDENTIFICATION.:
A) COMPLIES IR SPECTRUM AS PER COMPANY SPECS.

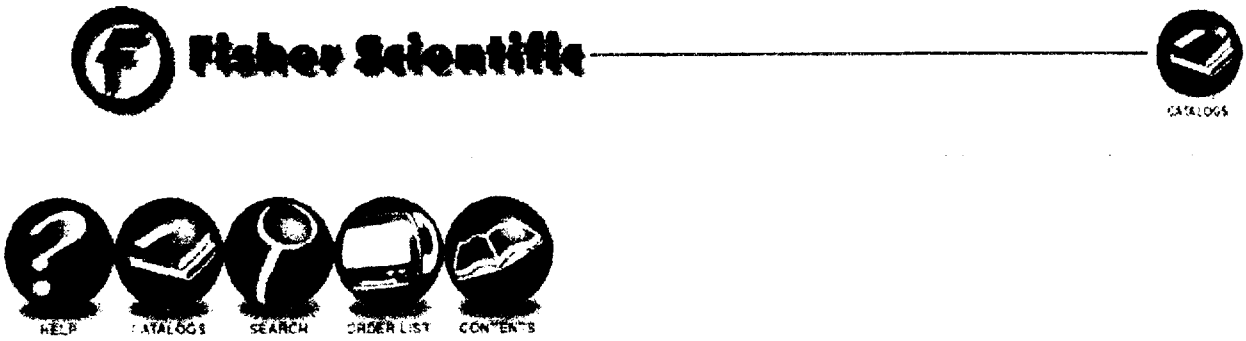
PASSES.: _____ **FAILS.:** _____

COMMENTS.: FULL NAME.: DIMERCAPTO-1-PROPANESULFONIC ACID SODIUM SALT 2,3.

ANALYST SIGNATURE.: _____ **DATE.:** _____

PREPACK TEST.: _____ **DATE.:** _____ **INITIAL.:** _____

RETEST.: _____ **DATE.:** _____ **INITIAL.:** _____



Use your web browser's "Back" key to return to previous topic.

MATERIAL SAFETY DATA SHEET

Dl-2,3-Dimercapto-1-Propanesulfonic Acid, Sodium Salt, Monohydrate 95%
02225

**** SECTION 1 - CHEMICAL PRODUCT AND COMPANY IDENTIFICATION ****

MSDS Name: Dl-2,3-Dimercapto-1-Propanesulfonic Acid, Sodium Salt, Monohydrate B

Synonyms:
DMPS
Company Identification: Acros Organics N.V.
One Reagent Lane
Fairlawn, NJ 07410
For information in North America, call: 800-ACROS-01
For emergencies in the US, call CHEMTREC: 800-424-9300
For emergencies in the US, call CHEMTREC: 800-424-9300

**** SECTION 2 - COMPOSITION, INFORMATION ON INGREDIENTS ****

CAS#	Chemical Name	%	EINECS#
4076-02-2	Dl-2,3-Dimercapto-1-Propanesulfonic Acid, Sodium Salt Monohydrate	95%	223-796-3

**** SECTION 3 - HAZARDS IDENTIFICATION ****

EMERGENCY OVERVIEW

Appearance: white.
Caution! Air sensitive. The toxicological properties of this material have not been fully investigated.
Target Organs: None known.

Potential Health Effects

Eye:
No information regarding eye irritation and other potential effects was found.

Skin:
No information regarding skin irritation and other potential effects was found.

Ingestion:
The toxicological properties of this substance have not been fully investigated.

Inhalation:
The toxicological properties of this substance have not been fully investigated. Inhalation of dust may cause respiratory tract

irritation.

Chronic:
Not available.

**** SECTION 4 - FIRST AID MEASURES ****

Eyes:
Flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower lids. Get medical aid immediately.

Skin:
Get medical aid immediately. Flush skin with plenty of soap and water for at least 15 minutes while removing contaminated clothing and shoes.

Ingestion:
If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.

Inhalation:
Get medical aid immediately. Remove from exposure to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.

Notes to Physician:
Treat symptomatically and supportively.

**** SECTION 5 - FIRE FIGHTING MEASURES ****

General Information:
As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear.

Extinguishing Media:
Use agent most appropriate to extinguish fire.

Autoignition Temperature: Not available.

Flash Point: Not available.

NFPA Rating: Not published.

Explosion Limits, Lower: Not available.
Upper: Not available.

**** SECTION 6 - ACCIDENTAL RELEASE MEASURES ****

General Information: Use proper personal protective equipment as indicated in Section 8.

Spills/Leaks:
Sweep up or absorb material, then place into a suitable clean, dry, closed container for disposal. Avoid generating dusty conditions.

**** SECTION 7 - HANDLING and STORAGE ****

Handling:
Wash thoroughly after handling. Use only in a well ventilated area. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation.

Storage:
Store in a cool, dry place. Keep container closed when not in use.

**** SECTION 8 - EXPOSURE CONTROLS, PERSONAL PROTECTION ****

Engineering Controls:
Use adequate ventilation to keep airborne concentrations low.

Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
DI-2,3-Dimercapto-1-Propanesulfonic Acid, Sodium Salt Monohydrate	none listed	none listed	none listed

OSHA Vacated PELs:
DI-2,3-Dimercapto-1-Propanesulfonic Acid, Sodium Salt Monohydrate:
No OSHA Vacated PELs are listed for this chemical.

Personal Protective Equipment

Eyes:
Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133.

Skin:
Wear appropriate protective gloves to prevent skin exposure.

Clothing:
Wear appropriate protective clothing to prevent skin exposure.

Respirators:
Follow the OSHA respirator regulations found in 29CFR 1910.134. Always use a NIOSH-approved respirator when necessary.

**** SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES ****

Physical State:	Solid
Appearance:	white
Odor:	None reported.
pH:	5.0 0.5
Vapor Pressure:	Not available.
Vapor Density:	Not available.
Evaporation Rate:	Not available.
Viscosity:	Not available.
Boiling Point:	@ 760.00mm Hg
Freezing/Melting Point:	229 deg C
Decomposition Temperature:	Not available.
Solubility:	Not available.
Specific Gravity/Density:	Not available.
Molecular Formula:	C3H7O3S3Na.H2O
Molecular Weight:	228.28

**** SECTION 10 - STABILITY AND REACTIVITY ****

Chemical Stability:
Stable under normal temperatures and pressures.
Conditions to Avoid:
Not available.
Incompatibilities with Other Materials:
Strong oxidizing agents.
Hazardous Decomposition Products:
Carbon monoxide, oxides of sulfur, carbon dioxide, sodium oxide.
Hazardous Polymerization: Has not been reported.

**** SECTION 11 - TOXICOLOGICAL INFORMATION ****

RTECS#:
CAS# 4076-02-2: TZ6420000
LD50/LC50:
Not available.
Carcinogenicity:
DI-2,3-Dimercapto-1-Propanesulfonic Acid, Sodium Salt Monohydrate -
Not listed by ACGIH, IARC, NIOSH, NTP, or OSHA.
Epidemiology:
No data available.
Teratogenicity:
No data available.
Reproductive Effects:
No data available.
Neurotoxicity:
No data available.
Mutagenicity:
No data available.
Other Studies:
No data available.

**** SECTION 12 - ECOLOGICAL INFORMATION ****

Ecotoxicity:
Not available.
Environmental Fate:
Not available.
Physical/Chemical:
Not available.
Other:
Not available.

**** SECTION 13 - DISPOSAL CONSIDERATIONS ****

Dispose of in a manner consistent with federal, state, and local regulations.
RCRA D-Series Maximum Concentration of Contaminants: Not listed.
RCRA D-Series Chronic Toxicity Reference Levels: Not listed.
RCRA F-Series: Not listed.
RCRA P-Series: Not listed.
RCRA U-Series: Not listed.
Not listed as a material banned from land disposal according to RCRA.

**** SECTION 14 - TRANSPORT INFORMATION ****

US DOT
No information available
IMO
Not regulated as a hazardous material.
IATA
Not regulated as a hazardous material.
RID/ADR
Not regulated as a hazardous material.
Canadian TDG
No information available.

**** SECTION 15 - REGULATORY INFORMATION ****

US FEDERAL
TSCA
CAS# 4076-02-2 is not listed on the TSCA inventory.
It is for research and development use only.
Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.
Section 12b
None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.
SARA
Section 302 (RQ)
None of the chemicals in this material have an RQ.
Section 302 (TPQ)
None of the chemicals in this product have a TPQ.
Section 313
No chemicals are reportable under Section 313.
Clean Air Act:
This material does not contain any hazardous air pollutants.
This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.
Clean Water Act:
None of the chemicals in this product are listed as Hazardous Substances under the CWA.
None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA:
None of the chemicals in this product are considered highly hazardous by OSHA.
STATE
Not present on state lists from CA, PA, MN, MA, FL, or NJ.

California No Significant Risk Level:
None of the chemicals in this product are listed.
European/International Regulations
European Labeling in Accordance with EC Directives
Hazard Symbols: Not available.
Risk Phrases:
Safety Phrases:
S 24/25 Avoid contact with skin and eyes.
WGK (Water Danger/Protection)
CAS# 4076-02-2:
Canada
None of the chemicals in this product are listed on the DSL/NDSL list.
WHMIS: Not available.
CAS# 4076-02-2 is not listed on Canada's Ingredient Disclosure List.
Exposure Limits

**** SECTION 16 - ADDITIONAL INFORMATION ****

MSDS Creation Date: 3/07/1992 Revision #3 Date: 9/11/1997

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no way shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

Back to product information.

...o relatively non-toxic thiocyanate, and thus ...s the detoxification of cyanide.

...l dosage regimen in adults is 300 mg of so... ...rite (10 mL of a 3% solution) administered ...venous injection over 3 minutes followed by ...f sodium thiosulphate (50 mL of a 25% so... ...25 mL of a 50% solution) administered in... ...ly over a period of about 10 minutes. A ...d dosage regimen in children is 0.15 to ...L per kg body-weight of a 3% solution of so... ...rite (approximately 4.5 to 10.0 mg per kg) ...d by 1.65 mL per kg of a 25% solution of ...thiosulphate (412.5 mg per kg). The meth... ...obin concentration should not exceed 30 to ...f symptoms of cyanide toxicity recur, it has ...gested that the injections of nitrite and thi... ...te may be repeated after 30 minutes at half ...al doses.

...thiosulphate is used as an isotonic 4% solu... ...the management of extravasation of mustine ...been tried in the management of extravasa... ...some other antineoplastic agents (although ...a contentious area, see p.516).

...thiosulphate has antifungal properties and ...en used topically in the treatment of pityriasis ...olor; the usual treatment of this infection is ...ed on p.397. Sodium thiosulphate and mag... ...thiosulphate are included in mixed prepara... ...or a variety of disorders.

...d acceptable daily intake of sodium thiosulphate as ...to 700 µg per kg body-weight.

...ces.

...WHO. Evaluation of certain food additives and contami... ...twenty-second report of the joint FAO/WHO expert ...ee on food additives. *WHO Tech Rep Ser 631* 1978.

...WHO. Evaluation of certain food additives and contami... ...twenty-seventh report of the joint FAO/WHO expert ...ee on food additives. *WHO Tech Rep Ser 696* 1983.

...port of sodium thiosulphate given by intravenous in... ...ducing the incidence of nephrotoxicity associated ...peritoneal cisplatin, see under Adverse Effects in ..., p.552.

...te poisoning. Sodium thiosulphate has been admin... ...in the treatment of bromate poisoning[1,2] although its ...efficacy is unclear.[3] Sodium thiosulphate is thought to ...educing bromate to the less toxic bromide ion, but ex... ...al evidence is lacking.[3] However, the high morbidity ...tality associated with bromate poisoning may justify ...of this relatively innocuous compound in some clini... ...mstances.[4]

...M, *et al.* Bromate poisoning from ingestion of profession... ...care neutralizer. *Clin Pharm* 1988; **7**: 66–70.

...berg R, *et al.* Bromate poisoning. *J Pediatr* 1989; **114**: ...

...ree NE, Kearney TE. Sodium thiosulfate unproven as ...e antidote. *Clin Pharm* 1988; **7**: 570, 572.

...n CE. Sodium thiosulfate unproven as bromate antidote. ...Pharm 1988; **7**: 572.

...rations

...d preparations are listed below; details are given in Part 3.

...d Preparations

...: Sodium Thiosulphate Injection;
...: Sodium Thiosulfate Injection.

...ary Preparations

...sulfène; *Ger.:* S-hydril†.

...gredient preparations. *Aust.:* Schwefelbad Dr Klop... ...: ITC; *Canad.:* Adasept; *Fr.:* Artérase; Désintex; Désin... ...tile; Désintex-Choline; Désintex-Pentazol†; Digestal†; ...rbine; Rhino-Sulfuryl; Sulfo-Thiorine Pantothénique†; ...yl; *Ger.:* Corti Jaikal; Jaikal; Jodcalcium-POS†; Phera... ...chwefelbad-Dr. Klopfer N; *Ital.:* Istaglobina†; Salicilato At... ...Asat†; Zeta-Bat; *S.Afr.:* Tripac-Cyano; *Spain:* Artro Gamma ...Artro Gamma Vit B1†; Artrochemi†; Nacient Sulf†; Yodo ...Colir†; *Switz.:* Blephamide; Sébo Lotion; Sulfo-Balmiral†; ...ol; *USA:* Cyanide Antidote Package; Komed†; Tinver.

...cimer (1058-k)

...r (*BAN, USAN, rINN*).

...A; DMSA. *meso*-2,3-Dimercaptosuccinic acid; (*R**,*S**)-...ercapto-butanedioic acid.

...O$_4$S$_2$ = 182.2.

...304-55-2.

Adverse Effects and Precautions

Succimer may cause gastro-intestinal disorders, skin rashes, increases in serum transaminase, flu-like symptoms, drowsiness, and dizziness. Succimer should be used with caution in patients with impaired renal function or a history of hepatic disease.

Pharmacokinetics

Following oral administration succimer is rapidly but incompletely absorbed. It undergoes rapid and extensive metabolism and is excreted mainly in the urine with small amounts excreted in the faeces and via the lungs.

References.

1. Dart RC, *et al.* Pharmacokinetics of meso-2,3-dimercaptosuccinic acid in patients with lead poisoning and in healthy adults. *J Pediatr* 1994; **125**: 309–16.

Uses and Administration

Succimer is a chelating agent structurally related to dimercaprol (see p.980). It forms water-soluble chelates with heavy metals and is used in the treatment of acute poisoning with lead, arsenic, or mercury.

Succimer may also be used in the management of cystinuria. Succimer, labelled with a radionuclide, is used in nuclear medicine.

In the treatment of lead poisoning, succimer is given by mouth in a suggested dose of 10 mg per kg body-weight or 350 mg per m^2 body-surface area every 8 hours for 5 days then every 12 hours for an additional 14 days. The course of treatment may be repeated if necessary, usually after an interval of not less than 2 weeks. The management of lead poisoning, including the use of succimer, is discussed under Lead, Treatment of Adverse Effects, p.1720.

Reviews.

1. Anonymous. Succimer—an oral drug for lead poisoning. *Med Lett Drugs Ther* 1991; **33**: 78.
2. Mann KV, Travers JD. Succimer, an oral lead chelator. *Clin Pharm* 1991; **10**: 914–22.

Extracorporeal administration. Extracorporeal infusion of succimer into the arterial blood line during haemodialysis, a procedure known as extracorporeal regional complexing haemodialysis, produced a substantial clearance of mercury in an anuric patient following intoxication with inorganic mercury.[1] Clearance was approximately ten times greater than that achieved with haemodialysis following intramuscular administration of dimercaprol.

1. Kostyniak PJ, *et al.* Extracorporeal regional complexing haemodialysis treatment of acute inorganic mercury intoxication. *Hum Toxicol* 1990; **9**: 137–41.

Preparations

Names of preparations are listed below; details are given in Part 3.

Proprietary Preparations

USA: Chemet.

Trientine Dihydrochloride (13377-a)

Trientine Dihydrochloride (*BAN, rINNM*).

MK-0681; Trien Hydrochloride; Trientine Hydrochloride (*USAN*); Triethylenetetramine Dihydrochloride. 2,2'-Ethylenedi-iminobis(ethylamine) dihydrochloride; *N,N'*-bis(2-Aminoethyl)-1,2-ethanediamine dihydrochloride.

$C_6H_{18}N_4.2HCl$ = 219.2.

CAS — 112-24-3 (trientine); 38260-01-4 (trientine dihydrochloride).

Pharmacopoeias. In *US.*

A white to pale yellow crystalline powder. Freely **soluble** in water; soluble in methyl alcohol; slightly soluble in alcohol; practically insoluble in chloroform and in ether. A 1% solution in water has a pH of 7.0 to 8.5. **Store** under an inert gas in airtight containers at 2° to 8°. Protect from light.

Adverse Effects and Precautions

Trientine dihydrochloride may cause iron deficiency. If iron supplements are given an interval of at least 2 hours between the administration of a dose of trientine and iron has been recommended. Recurrence of symptoms of systemic lupus erythematosus has been reported in a patient who had previously reacted to penicillamine.

Uses and Administration

Trientine dihydrochloride is a copper chelating agent used in a similar way to penicillamine in the treatment of Wilson's disease. It tends to be used in patients intolerant to penicillamine. For a discussion of the management of Wilson's disease see p.992.

Trientine dihydrochloride is administered by mouth, preferably on an empty stomach. The usual initial dose is 750 mg to 1250 mg daily in 2 to 4 divided doses increasing to a maximum of 2 g daily if required. In children, the usual initial dose is 500 to 750 mg daily increasing to a maximum of 1.5 g daily if required.

Preparations

Names of preparations are listed below; details are given in Part 3.

Official Preparations

USP 23: Trientine Hydrochloride Capsules.

Proprietary Preparations

USA: Syprine.

Unithiol (1059-a)

DMPS; Unitiol; Sodium 2,3-dimercaptopropanesulphonate.

$C_3H_7NaO_3S_3$ = 210.3.

CAS — 4076-02-2.

Unithiol is a chelating agent structurally related to dimercaprol (see p.980). It is water soluble and reported to be less toxic than dimercaprol. Unithiol is used in the treatment of poisoning by heavy metals including arsenic, lead, inorganic and organic mercury compounds, and chromium. It may be less effective in cadmium poisoning.

Unithiol is given by mouth in doses of 100 mg three times daily. It has also been administered parenterally.

Reviews.

1. Aposhian HV. DMSA and DMPS—water soluble antidotes for heavy metal poisoning. *Ann Rev Pharmacol Toxicol* 1983; **23**: 193–215.
2. Hruby K, Donner A. 2,3-Dimercapto-1-propanesulphonate in heavy metal poisoning. *Med Toxicol* 1987; **2**: 317–23.

Lead poisoning. Unithiol has been tried in twelve children with chronic lead poisoning.[1] It reduced lead concentrations in blood but did not affect the concentrations of copper or zinc in plasma. During treatment the urinary excretion of lead, copper, and zinc was increased.

The usual chelating agents used in the management of lead poisoning are discussed on p.1720.

1. Chisolm JJ, Thomas DJ. Use of 2,3-dimercaptopropane-1-sulfonate in treatment of lead poisoning in children. *J Pharmacol Exp Ther* 1985; **235**: 665–9.

Mercury poisoning. Administration of unithiol 100 mg twice daily by mouth for a maximum of 15 days enhanced urinary elimination of mercury in 7 patients with mercury poisoning.[1] The urinary elimination of copper and zinc was also increased in most patients and two developed skin rashes. Unithiol, 50 mg per 10 kg body-weight by intramuscular injection three times a day reducing to 50 mg per 10 kg once a day by the third day of treatment, effectively reduced the half-life of mercury in the blood following poisoning with methylmercury.[2]

1. Mant TGK. Clinical studies with dimercaptopropane sulphonate in mercury poisoning. *Hum Toxicol* 1985; **4**: 346.
2. Clarkson TW, *et al.* Tests of efficacy of antidotes for removal of methylmercury in human poisoning during the Iraq outbreak. *J Pharmacol Exp Ther* 1981; **218**: 74–83.

Wilson's disease. Unithiol 200 mg twice daily[1] was used successfully to maintain cupriuresis in a 13-year-old boy with Wilson's disease after he developed systemic lupus during treatment with penicillamine and with trientine dihydrochloride, which are two of the usual agents used in Wilson's disease (see p.992). Unithiol was started in two similar patients[1] but both withdrew from treatment, one because of fever and a fall in leucocyte count following a test dose and the other because of intense nausea and taste impairment.

1. Walshe JM. Unithiol in Wilson's disease. *Br Med J* 1985; **290**: 673–4.

Preparations

Names of preparations are listed below; details are given in Part 3.

Proprietary Preparations

Ger.: Dimaval; Mercuval.

[redacted]

2, 3-DIMERCAPTO-1-PROPANE SULFONIC ACID (DMPS) IN THE TREATMENT OF HEAVY METAL POISONING

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February 17 through March 28, 1997

2, 3-DIMERCAPTO-1-PROPANE SULFONIC ACID (DMPS) IN THE TREATMENT OF HEAVY METAL POISONING

Introduction

The use of chelating agents, such as BAL (British Anti-Lewisite), calcium disodium EDTA, and penicillamine in the treatment of human exposure to toxic metals has been well known and accepted (1, 2, 3). However, these chelating agents have limited therapeutic efficacies and undesirable side effects. In the quest for therapeutically more potent and less toxic metal-binding agents to use in the treatment of heavy metal poisoning, scientists have found that the sodium salt of 2, 3-dimercapto-1-propanesulfonic acid (DMPS) is an example of such a compound (2, 4). It is a water-soluble chemical analog of dimercaprol (BAL) but is less toxic. It is administered parenterally and is also effective when given orally. It has been used as an official drug in the Soviet Union since 1958 as Unithiol®. DMPS has been approved by the German Food and Drug Administration (FDA) and is manufactured by Heyl & Co. in Berlin, Germany as Dimaval®. However, it is a relatively new antidote, especially to this country; it is being used in the United States as an investigational drug (2).

Chemical Properties

Structurally, DMPS is related to BAL (Figure 1) (1, 2, 3). It is a sulfonic acid salt with two free sulfhydryl groups (SH-) which form complexes with heavy metals, such as mercury

(Hg), cadmium (Cd), arsenic (As), lead (Pb), copper (Cu), silver (Ag), tin (Sn), and others. It is soluble in water and can be administered parenterally as well as orally. DMPS has a distinct odor; thus, it is recommended to administer the drug in ice cold orange juice or in ice cold apple sauce (3). In addition, stability studies on DMPS are currently not available, but it is considered to be very stable and not readily oxidized during pre-use storage.

Therapeutic Use

As mentioned earlier, DMPS is used in the treatment of poisoning in humans by heavy metals including mercury, arsenic, and lead (Table 1) (2, 6, 8, 9, 10). It is registered with the German FDA for the treatment of mercury poisoning and is in fact sold in Germany without the need of a prescription (2). It has also been used as a mercury challenge or diagnostic test for mercury exposure; it has been found as the ideal agent to detoxify patients that have suffered from mercury toxicity from dental amalgam fillings after the fillings have been removed (1, 5, 6). Moreover, DMPS has been reported to be useful in Wilson's disease in which tissue levels of copper are high. It is given by mouth as a single dose of 300 mg or 100 mg three times daily for as long as 15 days. In the treatment of lead poisoning in children an oral dose of 200 mg to 400 mg of DMPS per meter squared body surface area per day was used effectively without observable adverse drug reactions (8). When given parenterally, 5 mg per kg body weight three times a day was the recommended dose(7).

Pharmacokinetic Studies

DMPS has been extensively used in humans both in the Soviet Union and in Germany, and pharmacokinetic data after intravenous (IV) and oral (PO) administration of this drug are widely available (1, 2, 11, 12). Studies have shown that DMPS is distributed both extracellularly and to a smaller extent intracellularly (5, 11). Scientists made an assumption that if the drug appeared in the bile then it must have entered liver cells first, and experiments done in rats proved that DMPS does enter the liver cells in small amounts (5).

In the plasma, DMPS is found to be about 62.5% bound by protein, mainly albumin, via a disulfide linkage (5, 11, 12). This was elucidated by treating the isolated DMPS-albumin complex from the urine with dithiothreitol (DTT) to give back DMPS, the parent compound. The DMPS-albumin disulfide complex is quite stable and may prolong the heavy metal mobilizing activity of DMPS. As a matter of fact, the half-life of the parent compound was 1.8 hours; whereas, that of altered DMPS was 20 hours.

DMPS is metabolized rapidly and is eliminated in the kidney and bile (4, 5, 11, 12). Hurlbut, *et. al.* (1994) demonstrated that only about 12% or 9%, respectively, of the DMPS concentration detected in the urine is presented as the parent drug after fifteen minutes of IV or PO administration of DMPS, suggesting that the majority of the DMPS in the urine were the metabolites or the oxidized forms of the drug (12). In humans, DMPS is biotransformed or oxidized to acyclic polymeric disulfides (which constitute only 0.5% of the total DMPS disulfides) in the liver and cyclic polymeric disulfides (97% of the total DMPS disulfides) in the bile (Figure 2) (2, 5, 12). The amount of altered or unaltered DMPS was determined

using an assay that employed the chemical known as bromobimane to react with the thiols (Figure 3) (5, 12). Neither bromobimane nor DMPS has a fluorescence, but bromobimane would react with DMPS to form a highly fluorescent bimane derivative. The resulting compound is then analyzed using the technique of HPLC (High-Performance Liquid Chromatography) to detect unaltered molecules. The value of the altered or biotransformed molecules of the drug is then determined by subtracting the value of experimentally determined unaltered forms from the value of experimentally determined total DMPS (5).

Nevertheless, the disulfide group and certainly the sulfonic group are very poor chelators, especially of mercury or lead (12). The two sulfhydryl groups of DMPS are necessary for chelation. DMPS disulfides appear to be transported and reduced to DMPS within the renal tubules in the kidney where chelation of mercury by DMPS increases mercury excretion in the urine.

Oral DMPS appears to be less effective; oral bioavailability of DMPS is about 60% (11). The half-life found for total DMPS in a study after IV administration was approximately 20 hours, which was considerably longer than the half-life of 9.5 hours found for total DMPS after oral administration to humans (11). These values may represent differences in the metabolites produced after oral and IV administration. Other pharmacokinetic parameters of the drug include an elimination half-life of 43 minutes, a volume of distribution (Vd) of 160 ml/kg, and a clearance (CL) of 2.6 ml/min/kg (1, 11).

Toxicities

DMPS is a relatively safe drug and has been used innocuously in Europe for many years (1). In the studies done on DMPS at a dose of 5 mg/kg, some patients developed allergic reactions to the drug. This is usually because the patients have a history of allergies. No anaphylactic shock was seen. Other common side effects experienced by some patients were mild and include nausea, weakness, vertigo, and itching skin. No nephrotoxicity was observed. It also exhibited no mutagenic or teratogenic effects (1). When the dosage was increased to 100 mg/kg, the increased effectiveness was noted, but necrotization and ulcerations often occurred at the site of the subcutaneous (SC) or IV injection. However, when injected IV, DMPS should be given over a five minute period since hypotensive effects are possible when it is given parenterally as a bolus (2).

DMPS vs. Other Chelating Agents

In the treatment of heavy metal poisoning, BAL and calcium disodium EDTA are becoming obsolete. Water-soluble chelating agents like DMSA (succimer, Chemet®) and DMPS are therapeutically more potent and less toxic (1, 2, 5). When compared with D-penicillamine and N-acetyl DL-penicillamine, DMPS was the most effective for clearing mercury from the blood (6). It is more advantageous than DMSA since it has been extensively used in the Soviet Union and in Germany, and capsules for oral use as well as parenteral preparations of DMPS are available. DMSA, on the other hand, is only available

orally, thus, pharmacokinetics of DMSA are somewhat limited. Additionally, DMPS does not cause a redistribution of Hg to the brain like calcium disodium EDTA can. DMPS is more specific than calcium disodium EDTA; at diagnostic doses, DMPS would not be expected to increase the urinary excretion of essential trace elements such as copper and zinc. DMPS is able to enter cells to a certain extent and thus is intermediate in its toxicity. Comparatively, DMSA is the least toxic of the dimercapto chelating agents and has the highest LD_{50} since it does not get into cells (Table 1).

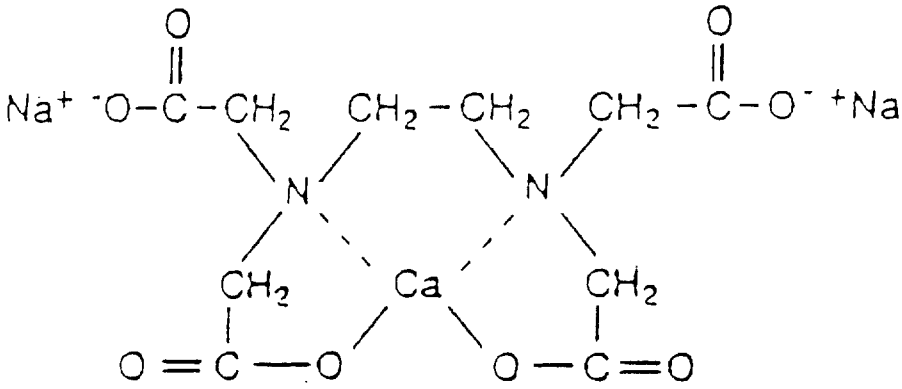
Conclusion

In retrospect, it appears that there is no better chelating agent than DMPS in treating heavy metal poisoning. None of the other chelating agents including DMSA, BAL, penicillamine, or calcium disodium EDTA is as therapeutically diverse and potent in detoxifying patients of heavy metals as DMPS. Other chelating agents are more toxic to use; whereas, DMPS is a relatively safe drug. Thus, in the treatment of heavy metal poisoning 2, 3-dimercapto-1-propane sulfonic acid is the recommended choice.

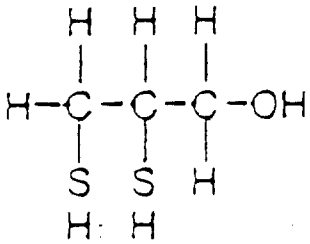
Figure 1

Chemical formulas for chelating agents used for treating heavy metal poisoning of humans

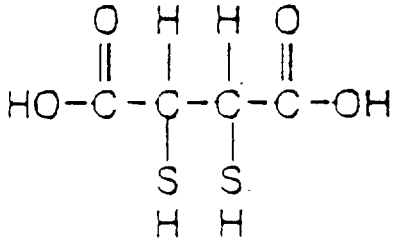
(From Reference #2)



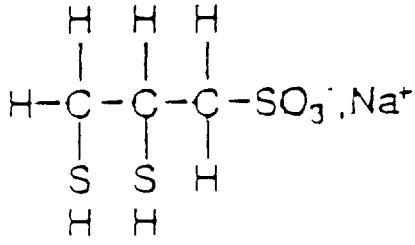
$CaNa_2EDTA$
(Edetate Calcium Disodium)



Dimercaprol
(British Antilewisite, BAL)



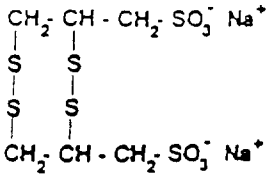
DMSA
(Meso-Dimercapto Succinic Acid)
Succimer



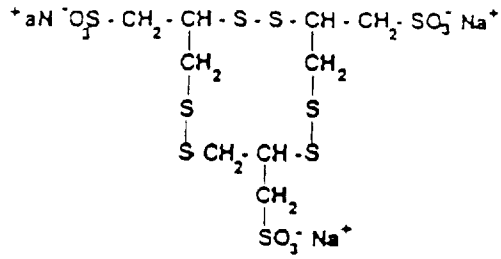
DMPS
(2,3-Dimercapto-1-Propane-Sulfonic Acid, Na Salt)
Dimaval

Figure 2

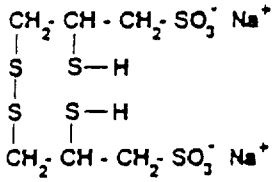
Proposed Structures of the human urinary metabolites of DMPS (From Reference # 12)



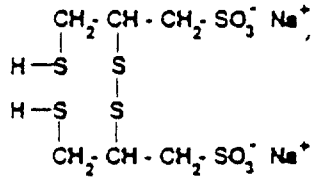
cyclic polymeric disulfide (dimer)



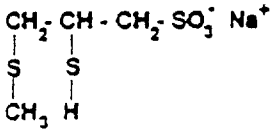
cyclic polymeric disulfide (trimer)



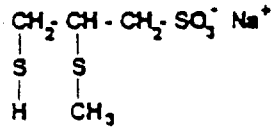
acyclic disulfide (dimer 1)



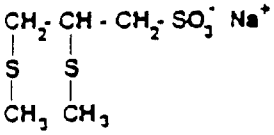
acyclic disulfide (dimer 2)



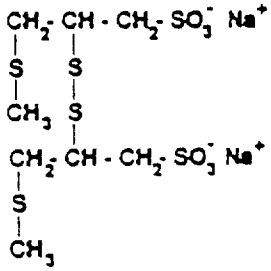
3 - monomethyl DMPS



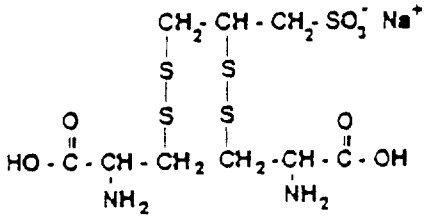
2 - monomethyl DMPS



S,S-dimethyl DMPS



monomethyl DMPS disulfide



DMPS-cysteine (1:2) mixed disulfide

Figure 3

Proposed Reactions of bromobimane with DMPS (From Reference #5)

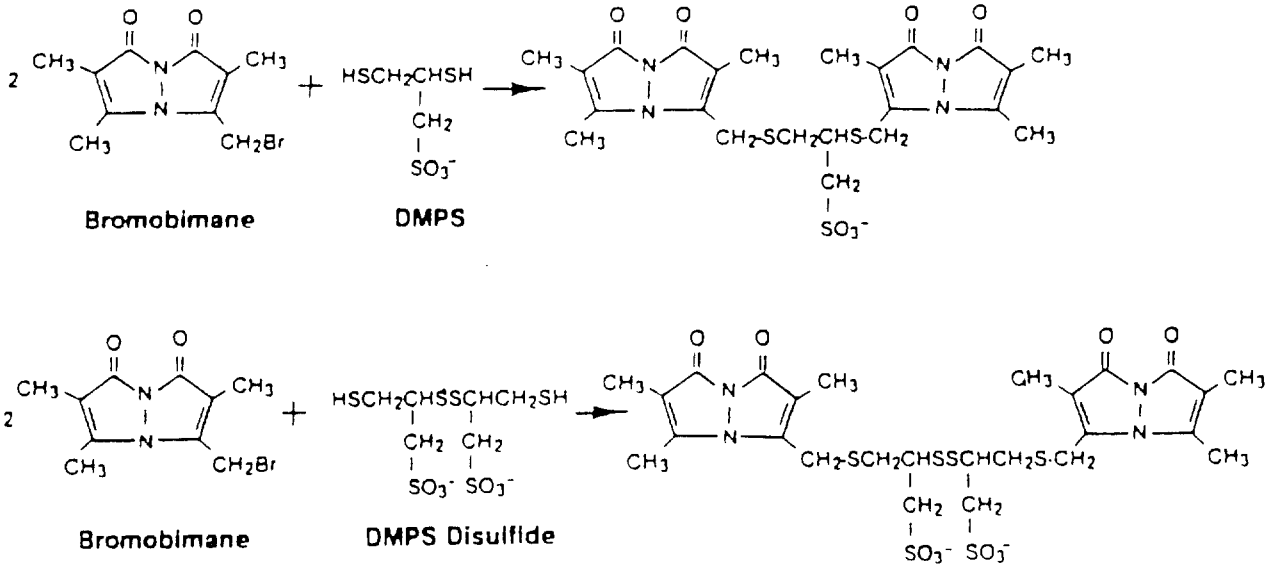


Table 1

Indications and Contraindications of chelating agents in heavy metal poisonings
(From Reference #2)

Metal*	**First Choice**	**Second Choice**	**Contraindications**
Hg metal	DMPS	DMSA	Dimercaprol
Hg inorganic	DMPS	DMSA	Dimercaprol
Hg organic	DMSA, DMPS		Dimercaprol
Pb	DMSA	DMPS	Dimercaprol, EDTA
As	DMPS, DMSA	Dimercaprol	Dimercaprol (?)
Cr	DMPS		
Sb	DMPS		
Transuranics	DTPA		

*Abbreviations: Hg= mercury; Pb= lead; As= arsenic; Cr=chromium; Sb=antimony.

Table 2

*LD_{50} Determination intraparenterally in mice (From Reference #5)

Compound	LD_{50} (mmol/kg)	95% confidence interval	Number of mice
BAL	1.48	1.11, 1.97	212
DMPA	0.82	0.80, 0.84	172
DMPS	6.53	5.49, 7.71	88
meso-DMSA	13.73	11.36, 15.22	164

*LD_{50} = median lethal dose.

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6 - include all references

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11. Hurlbut, K. M., Maiorino, R. M., Mayersohn, M., et. al.. Determination and Metabolism of Dithiol Chelating Agents XVI: Pharmacokinetics of 2, 3-Dimercapto-1-Propanesulfonate after Intravenous Administration to Human Volunteers. *The Journal of Pharmacology and Experimental Therapeutics*. (1994). 268(2): 662-668.

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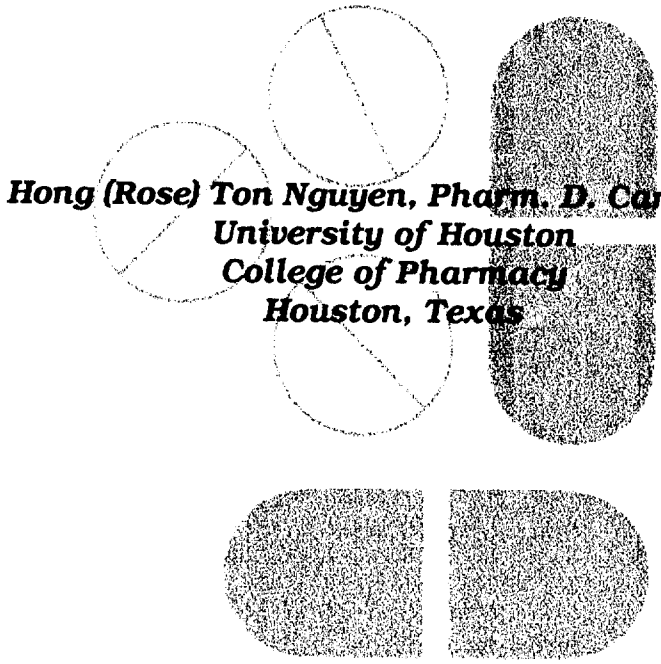
2, 3-DIMERCAPTO-1-PROPANE SULFONIC ACID (DMPS) IN THE TREATMENT OF HEAVY METAL POISONING



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2, 3-DIMERCAPTO-1-PROPANE SULFONIC ACID (DMPS) IN THE TREATMENT OF HEAVY METAL POISONING

Introduction

The use of chelating agents, such as BAL (British Anti-Lewisite), calcium disodium EDTA, and penicillamine in the treatment of human exposure to toxic metals has been well known and accepted (1, 2, 3). However, these chelating agents have limited therapeutic efficacies and undesirable side effects. In the quest for therapeutically more potent and less toxic metal-binding agents to use in the treatment of heavy metal poisoning, scientists have found that the sodium salt of 2, 3-dimercapto-1-propanesulfonic acid (DMPS) is an example of such a compound (2, 4). It is a water-soluble chemical analog of dimercaprol (BAL) but is less toxic. It is administered parenterally and is also effective when given orally. It has been used as an official drug in the Soviet Union since 1958 as Unithiol®. DMPS has been approved by the German Food and Drug Administration (FDA) and is manufactured by Heyl & Co. in Berlin, Germany as Dimaval®. However, it is a relatively new antidote, especially to this country; it is being used in the United States as an investigational drug (2).

Chemical Properties

Structurally, DMPS is related to BAL (Figure 1) (1, 2, 3). It is a sulfonic acid salt with two free sulfhydryl groups (SH-) which form complexes with heavy metals, such as mercury (Hg), cadmium (Cd), arsenic (As), lead (Pb), copper (Cu), silver (Ag), tin (Sn), and others. It is soluble in water and can be administered parenterally as well as orally. DMPS has a distinct odor; thus, it is recommended to

administer the drug in ice cold orange juice or in ice cold apple sauce (3). In addition, stability studies on DMPS are currently not available, but it is considered to be very stable and not readily oxidized during pre-use storage.

Therapeutic Use

As mentioned earlier, DMPS is used in the treatment of poisoning in humans by heavy metals including mercury, arsenic, and lead (Table I) (2, 6, 8, 9, 10). It is registered with the German FDA for the treatment of mercury poisoning and is in fact sold in Germany without the need of a prescription (2). It has also been used as a mercury challenge or diagnostic test for mercury exposure; it has been found as the ideal agent to detoxify patients that have suffered from mercury toxicity from dental amalgam fillings after the fillings have been removed (1, 5, 6). Moreover, DMPS has been reported to be useful in Wilson's disease in which tissue levels of copper are high. It is given by mouth as a single dose of 300 mg or 100 mg three times daily for as long as 15 days. In the treatment of lead poisoning in children an oral dose of 200 mg to 400 mg of DMPS per meter squared body surface area per day was used effectively without observable adverse drug reactions (8). When given parenterally, 5 mg per kg body weight three times a day was the recommended dose(7).

Pharmacokinetic Studies

DMPS has been extensively used in humans both in the Soviet Union and in Germany, and pharmacokinetic data after intravenous (IV) and oral (PO) administration of this drug are widely available (1, 2, 11, 12). Studies have shown that DMPS is distributed both extracellularly and to a smaller extent

intracellularly (5, 11). Scientists made an assumption that if the drug appeared in the bile then it must have entered liver cells first, and experiments done in rats proved that DMPS does enter the liver cells in small amounts (5).

In the plasma, DMPS is found to be about 62.5% bound by protein, mainly albumin, via a disulfide linkage (5, 11, 12). This was elucidated by treating the isolated DMPS-albumin complex from the urine with dithiothreitol (DTT) to give back DMPS, the parent compound. The DMPS-albumin disulfide complex is quite stable and may prolong the heavy metal mobilizing activity of DMPS. As a matter of fact, the half-life of the parent compound was 1.8 hours; whereas, that of altered DMPS was 20 hours.

DMPS is metabolized rapidly and is eliminated in the kidney and bile (4, 5, 11, 12). Hurlbut, *et. al.* (1994) demonstrated that only about 12% or 9%, respectively, of the DMPS concentration detected in the urine is presented as the parent drug after fifteen minutes of IV or PO administration of DMPS, suggesting that the majority of the DMPS in the urine were the metabolites or the oxidized forms of the drug (12). In humans, DMPS is biotransformed or oxidized to acyclic polymeric disulfides (which constitute only 0.5% of the total DMPS disulfides) in the liver and cyclic polymeric disulfides (97% of the total DMPS disulfides) in the bile (Figure 2) (2, 5, 12). The amount of altered or unaltered DMPS was determined using an assay that employed the chemical known as bromobimane to react with the thiols (Figure 3) (5, 12). Neither bromobimane nor DMPS has a fluorescence, but bromobimane would react with DMPS to form a highly fluorescent bimane derivative. The resulting compound is then analyzed using the technique of HPLC (High-Performance Liquid Chromatography) to detect unaltered molecules. The value of the altered or biotransformed molecules of the drug is then determined by subtracting the value of experimentally determined unaltered forms from the value of experimentally determined total DMPS (5).

Nevertheless, the disulfide group and certainly the sulfonic group are very poor chelators, especially of mercury or lead (12). The two sulfhydryl groups of DMPS are necessary for chelation. DMPS disulfides appear to be transported and reduced to DMPS within the renal tubules in the kidney where chelation of mercury by DMPS increases mercury excretion in the urine.

Oral DMPS appears to be less effective; oral bioavailability of DMPS is about 60% (11). The half-life found for total DMPS in a study after IV administration was approximately 20 hours, which was considerably longer than the half-life of 9.5 hours found for total DMPS after oral administration to humans (11). These values may represent differences in the metabolites produced after oral and IV administration. Other pharmacokinetic parameters of the drug include an elimination half-life of 43 minutes, a volume of distribution (Vd) of 160 ml/kg, and a clearance (CL) of 2.6 ml/min/kg (1, 11).

Toxicities

DMPS is a relatively safe drug and has been used innocuously in Europe for many years (1). In the studies done on DMPS at a dose of 5 mg/kg, some patients developed allergic reactions to the drug. This is usually because the patients have a history of allergies. No anaphylactic shock was seen. Other common side effects experienced by some patients were mild and include nausea, weakness, vertigo, and itching skin. No nephrotoxicity was observed. It also exhibited no mutagenic or teratogenic effects (1). When the dosage was increased to 100 mg/kg, the increased effectiveness was noted, but necrotization and ulcerations often occurred at the site of the subcutaneous (SC) or IV injection. However, when injected IV, DMPS should be given over a five minute period since hypotensive effects are possible when it is given

parenterally as a bolus (2).

DMPS vs. Other Chelating Agents

In the treatment of heavy metal poisoning, BAL and calcium disodium EDTA are becoming obsolete. Water-soluble chelating agents like DMSA (succimer, Chemet®) and DMPS are therapeutically more potent and less toxic (1, 2, 5). When compared with D-penicillamine and N-acetyl DL-penicillamine, DMPS was the most effective for clearing mercury from the blood (6). It is more advantageous than DMSA since it has been extensively used in the Soviet Union and in Germany, and capsules for oral use as well as parenteral preparations of DMPS are available. DMSA, on the other hand, is only available orally, thus, pharmacokinetics of DMSA are somewhat limited. Additionally, DMPS does not cause a redistribution of Hg to the brain like calcium disodium EDTA can. DMPS is more specific than calcium disodium EDTA; at diagnostic doses, DMPS would not be expected to increase the urinary excretion of essential trace elements such as copper and zinc. DMPS is able to enter cells to a certain extent and thus is intermediate in its toxicity.

Comparatively, DMSA is the least toxic of the dimercapto chelating agents and has the highest LD_{50} since it does not get into cells (Table 2).

Conclusion

In retrospect, it appears that there is no better chelating agent than DMPS in treating heavy metal poisoning. None of the other chelating agents including DMSA, BAL, penicillamine, or calcium disodium EDTA is as therapeutically diverse and potent in detoxifying patients of heavy metals as DMPS. Other

chelating agents are more toxic to use; whereas, DMPS is a relatively safe drug. Thus, in the treatment of heavy metal poisoning

2, 3-dimercapto-1-propane sulfonic acid is the recommended choice.

Figure I

Chemical formulas for chelating agents used for treating heavy metal poisoning of humans (From Reference #2)

Figure 2

Proposed Structures of the human urinary metabolites of DMPS (From Reference # 12)

Figure 3

Proposed Reactions of bromobimane with DMPS (From Reference #5)

Table I

Indications and Contraindications of chelating agents in heavy metal poisonings
(From Reference #2)

Metal*	First Choice	Second Choice	Contraindications
Hg metal	DMPS	DMSA	Dimercaprol
Hg inorganic	DMPS	DMSA	Dimercaprol
Hg organic	DMSA, DMPS		Dimercaprol
Pb	DMSA	DMPS	Dimercaprol, EDTA
As	DMPS, DMSA	Dimercaprol	Dimercaprol (?)
Cr	DMPS		
Sb	DMPS		
Transuranics	DTPA		

*Abbreviations: Hg= mercury; Pb= lead; As= arsenic; Cr=chromium; Sb=antimony.

Table 2

*LD_{50} Determination intraparenterally in mice (From Reference #5)

Compound	LD_{50} (mmol/kg)	95% confidence interval	Number of mice
BAL	1.48	1.11, 1.97	212
DMPA	0.82	0.80, 0.84	172
DMPS	6.53	5.49, 7.71	88
meso-DMSA	13.73	11.36, 15.22	164

*LD_{50} = median lethal dose.

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Meso-dimercaptosuccinic acid (DMSA) and the sodium salt of 2,3-dimercapto-1-propanesulfonic acid (DMPS) are analogous in chemical structure to dimercaprol (BAL, British Anti-Lewisite). Dimercaprol was among the first therapeutically useful metal chelating agents and was developed originally as an anti-lewisite agent. Either DMSA or DMPS protects rabbits from the lethal systemic action of dichloro(2-chlorovinyl)arsine (29.7 mumols/kg, also known as lewisite. The analogs are active in this respect when given either sc or po. The stability of each of the three dimercapto compounds in distilled H2O, pH 7.0 at 24 degrees, has been examined for seven days. DMSA retained 82% of its mercapto groups, but no titratable mercapto groups remained in the DMPS or BAL solutions. At pH 5.0, however, there was no striking difference in the stability of the three dimercapto compounds (78-87%) over a seven day period. DMSA and DMPS warrant further investigation as water soluble metal binding agents in both in vivo and in vitro experiments.

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TITLE:	**Biological chelation: 2,3-dimercapto-propanesulfonic acid and meso-dimercaptosuccinic acid.**
AUTHOR:	**Aposhian HV**
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NLM CIT. ID:	**82280847**
ABSTRACT:	**Water soluble analogs of British Anti-Lewisite that are active orally and less toxic than BAL are now available. These agents are 2,3-dimercapto-1-propanesulfonic acid and meso-dimercaptosuccinic acid. Evidence for their effectiveness in preventing the lethal effects of sodium arsenite in mice and lewisite in rabbits is presented. These analogs can be expected to replace BAL in the treatment of heavy metal poisoning.**
MAIN MESH SUBJECTS:	**Chelating Agents/*PHARMACOLOGY** **Dimercaprol/*ANALOGS & DERIVATIVES/PHARMACOLOGY** **Succimer/*PHARMACOLOGY** **Sulfhydryl Compounds/*PHARMACOLOGY** **Unithiol/*PHARMACOLOGY**
ADDITIONAL MESH SUBJECTS:	**Animal** **Arsenic/POISONING** **Cadmium Poisoning** **Lethal Dose 50** **Male** **Mice** **Penicillamine/ANALOGS & DERIVATIVES/PHARMACOLOGY** **Support, Non-U.S. Gov't**
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ANTI-LEWISITE ACTIVITY AND STABILITY OF MESO-DIMERCAPTOSUCCINIC ACID AND 2,3-DIMERCAPTO-1-PROPANESULFONIC ACID

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Summary

Meso-dimercaptosuccinic acid (DMSA) and the sodium salt of 2,3- dimercapto-1-propanesulfonic acid (DMPS) are analogous in chemical structure to dimercaprol (BAL, British Anti-Lewisite). Dimercaprol was among the first therapeutically useful metal chelating agents and was developed originally as an anti-lewisite agent. Either DMSA or DMPS protects rabbits from the lethal systemic action of dichloro(2-chlorovinyl)arsine (29.7μmols/kg, also known as lewisite. The analogs are active in this respect when given either sc or po. The stability of each of the three dimercapto compounds in distilled H_2O, pH 7.0 at 24°, has been examined for seven days. DMSA retained 82% of its mercapto groups, but no titratable mercapto groups remained in the DMPS or BAL solutions. At pH 5.0, however, there was no striking difference in the stability of the three dimercapto compounds (78-87%) over a seven day period. DMSA and DMPS warrent further investigation as water soluble metal binding agents in both in vivo and in vitro experiments.

British Anti-Lewisite (BAL, dimercaprol) was developed in the 1940's as an antidote to dichloro-(2-chlorovinyl)-arsine, commonly called lewisite (1,2). The lethal action of lewisite is believed to be the result of its combining with one or more sulfhydryl groups and thus inactivating essential sulfhydryl-containing enzymes (3). It is the arsenic in the lewisite molecule that reacts with sulfhydryl moieties.

At the time of its introduction into clinical medicine, BAL was considered by many to be the long sought, universal antidote for heavy metal poisoning. In subsequent years, however, less toxic and more specific metal binding agents have been sought and investigated. Some have met the criteria and standards necessary for clinical use. Others have not. For example, BAL glucoside was introduced (4) as a result of a search for water soluble and less toxic analogs of BAL. Although it was found to be less toxic than BAL for iv use, (probably because of its low lipoid solubility), it did not become established as a clinical agent because it is unstable chemically. Other compounds, which are less analogous in chemical structure, have replaced BAL for some of its more specific therapeutic uses. For example, D-penicillamine is used to mobilize and increase the excretion of copper in patients with Wilson's Disease (5). Its N-acetyl derivative is effective as a mercury antidote (6,7). BAL has remained, however, the drug of choice in the U.S. for the treatment of arsenic poisoning.

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Meso-dimercaptosuccinic acid (DMSA) (8) and the sodium salt of 2,3-dimercapto-1-propanesulfonic acid (DMPS) (9) are promising replacements for BAL. These compounds are very similar in chemical structure to BAL and are sometimes referred to as water soluble and/or orally-effective analogs of BAL. To our knowledge, however, the anti-lewisite activity of these two important chemical analogs has not been determined. Neither are any published data available concerning the stability of aqueous solutions of these dimercapto compounds. Evidence for the anti-lewisite activity and stability of DMPS and DMSA are presented in this paper.

Materials and Methods

Male New Zealand white stock rabbits weighing 2.5-3.5 kg were purchased from Dutchland Laboratories Inc., Denver, PA and Davidson Mill Farm, Jonesburg, NJ and caged individually. Food (Purina Rabbit Chow Brand 5322) and water were available ad libitum except in the case of those animals who received therapy orally. Animals receiving therapy po were fasted from 16 hrs prior to the first administration to 1 hr after the last administration on day one. On days two and three, animals were fasted from 1 hr prior to the morning administration to 1 hr after the evening administration, approximately 7 hours.

When dithiol therapy was given sc, the animals were anesthetized fifteen minutes before lewisite administration by administering im 0.50 ml of anesthetic solution per kg. The animals were anesthetized to reduce the pain expected to be caused by lewisite. Subsequently, it was observed that neither pain nor discomfort was apparent. Thus, anesthesia was not used in the experiments when dithiols were given po. The anesthetic solution was prepared by mixing 5 parts Ketamine HCl (100 mg/ml) and 1 part of Xylazine (100 mg/ml).

A 5 ml Gilson Pipetman was used to give the dithiols by mouth. The rabbit was placed in a short restraining box. The box was placed on its end so that the rabbit was in a vertical position with its head at the top. The Pipetman was filled with the desired volume of the drug solution. The plastic tip was gently inserted between the lips at one corner of the mouth and the liquid delivered slowly into the back of the rabbit's mouth. This method did not appear to cause any trauma or injury. It was easier and faster to perform than the use of polyethylene stomach tubes.

NaDMPS was a gift of Heyl and Co., Berlin. Since each molecule has a molecule of H_2O associated with it, a molecular weight of 228.2 was used in calculations. DMSA was a gift of Johnson and Johnson, Skillman, N.J. Both compounds were pharmaceutical grade purity. DMPS and DMSA were titrated with iodine in order to measure purity and mercapto content. By this criterion, each preparation was judged to be greater than 99% pure. The compounds when given by mouth were dissolved in water. In order to dissolve DMSA, the aqueous suspension was adjusted to pH 5.5 with NaOH. When given sc, the solutions were prepared the same way except that the compounds were dissolved in 0.9% NaCl-5% $NaHCO_3$. Unless otherwise stated, the concentrations of DMPS or DMSA were such that the rabbit received 1.0 ml of solution per kg of body weight, per administration. Dimercaprol Injection, USP (BAL in Oil Ampules) was a gift of Hynson, Wescott & Dunning, Baltimore, MD.

Lewisite was 97-99.6% pure as judged by NMR-spectroscopy as well as by iodine titration. Analysis by the former method also indicated that the forms of lewisite that were present were trans (97.7%), cis (1.7%) and dimers (0.5%). Lewisite is a hazardous material with which to work since it is a potent vesicant. All handling of lewisite was done in an extremely well

TABLE I

The Anti-Lewisite Activity of Meso-Dimercaptosuccinic Acid and 2,3,-Dimercapto-1-Propanesulfonate when given sc to rabbits

Group	µmols/kg	survive/start[a]	% survival
1	LEW[b] + --------	1/18	6
2	LEW + 75.0 DMSA[c]	12/12	100
3	LEW + 37.5 DMSA	6/6	100
4	LEW + 20.0 DMSA	6/6	100
5	LEW + 10.0 DMSA	1/6	17
6	LEW + 75.0 DMPS[c]	10/12	83
7	LEW + 37.5 DMPS	5/6	83
8	LEW + 75.0 BAL[c]	8/12	67
9	LEW + 37.5 BAL	3/6	50

[a] In the tables of this paper, the data represent the combined results of a number of separate experiments. This was done to save space. The reason for the number of animals in some groups differing from the number in other groups of the same table is that very often the combined data are the result of from 2-3 separate experiments. Otherwise, the experiments were performed under identical conditions. The survival recorded in this table is that for 7 days after lewisite administration.

[b] Lewisite (29.7 µmols/kg) was given sc at time zero.

[c] All agents given sc except BAL, which was given im. Dimercapto compounds given at +1 min, +90 min, +180 min, +360 min after lewisite and at 8 a.m. and 4 p.m. on day 2 and 3. Administration of these amounts of dimercapto compound at the times cited above did not cause any fatalities in control animals that did not receive lewisite (data not shown).

[d] Pair-wise comparisons: $p < 0.0001$ for 1 vs 2; $p = 0.0001$ for 1 vs 3 and 1 vs 4; $p < 0.001$ for 1 vs 6 and 1 vs 7; $p = 0.001$ for 1 vs 8 and 0.01 for 1 vs 9.

vented chemical exhaust hood. Safety glasses and thick neoprene gloves were worn.

The stability of DMPS, DMSA or BAL was determined using iodometric titration. To 2.50 ml of a 0.10 M dimercapto solution, 10 drops of starch indicator solution were added. The solution was titrated using 0.025 N iodine solution until the blue color appeared and persisted for at least 10 sec.

Results

Anti-Lewisite Activity

The data of Table 1 clearly show that both DMSA and DMPS have anti-lewisite activity when given subcutaneously. As little as 20μmols/DMSA/kg administered sc, according to the stated regimen, protects against the lethal actions of lewisite. Thus, DMSA and DMPS are analogous to BAL not only in chemical structure but also with respect to anti-lewisite activity. In addition, DMSA and DMPS have anti-lewisite activity when given orally (Table 2).

TABLE II

Meso-Dimercaptosuccinic Acid or 2,3-Dimercapto-1-Propanesulfonate is effective, when given by mouth, in protecting rabbits against the lethal effects of Lewisite

Group	μmols/kg	survive/start	% survival
1	LEW[a] + -------[b]	0/12	0
2	LEW + 400 DMSA[b]	5/6	83
3	LEW + 200 DMSA[b]	4/6	67
4	LEW + 400 DMPS[b]	6/6	100
5	LEW + 200 DMPS[b]	4/6	67
6	LEW + -------[c]	1/6	17
7	LEW + DMSA[c]	4/6	67
8	LEW + DMPS[c]	1/6	17

[a] Lewisite (29.7 μmols/kg) was given sc at time zero.

[b] Dimercapto compounds given po at -45, -2, +90 and +300 min. after lewisite and 8 a.m. and 4 p.m. on day 2 and 3. No fatalities occurred in control animals that received these amount of dimercapto compound, po, (but no lewisite) at the times cited above. Survival was followed and recorded for 7 days after lewisite administration.

[c] Dimercapto compounds given po as follows: 400μmols of dimercapto compound /kg at 5 min before lewisite, and 200μmols/kg at each of the following times after lewisite: 1 hr., 2.5 hrs. and 5 hrs. on the first day plus 8 a.m. and 4 p.m. on day 2 and 3.

[d] For pair-wise comparison: $p = 0.001$ for 1 vs 2; $p = 0.01$ for 1 vs 3; $p < 0.001$ for 1 vs 4 and $p = 0.01$ for 1 vs 5

Additional studies have demonstrated that a single po administration of DMSA (400 μmoles/kg) 15 min prior to lewisite was ineffective since only 1 of 6 animals survived for 7 days. In the experiments of Table 1 and 2, most of the rabbits that received lewisite and no dimercapto therapy died within 12 hrs. If animals died after receiving lewisite plus dimercapto therapy, they usually died between the first and fifth day of the experiment.

Stability Studies

The stabilities of DMSA, DMPS and BAL in 0.10M solutions at pH 5.0 and 7.0 were examined (Fig 1). The mercapto groups of these compounds, in aqueous solutions at pH 5.0, are stable (Fig 1). Even after 7 days at room temperature, from 78 to 87% of the mercapto groups remain titratable. At pH 7.0, however, the greater stability of DMSA is evident with 82% of the mercapto groups remaining after 7 days.

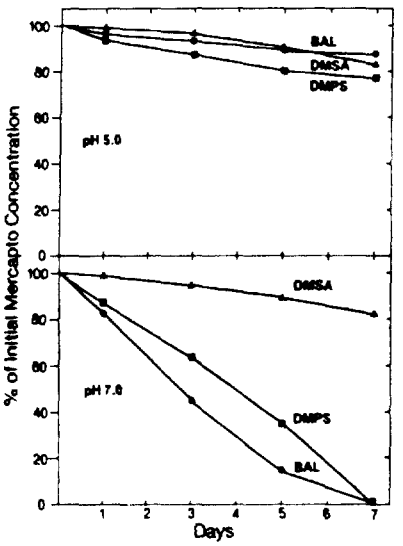

FIG. 1

Stability of DMSA, DMPS and BAL at pH 5.0 or 7.0. Aqueous solutions of each compound were prepared, adjusted to pH 5.0 or 7.0 and to a final concentration of 0.10mmol/ml. Solutions were prepared using double-distilled H_2O and maintained at 24°. Aliquots were removed at indicated times and the mercapto content determined. Each value shown is the average of two separate titrations.

By this time and under these conditions none of the mercapto groups of DMPS or BAL remained. Other studies (data not shown) indicated that DMSA, in a solution of 5% $NaHCO_3$ when either frozen for 4 days or frozen and thawed each day for 4 days retained 82% of its original mercapto groups. If similar solutions were held at 4° or 24° for 4 days, DMSA retained 76% and 69% of its mercapto groups, respectively. Although solutions of DMPS in 5% $NaHCO_3$ were stable (92-95% of original) when either frozen, or frozen and thawed each day for 7 days, after three days at room temperature no titratable mercapto groups were evident.

Discussion

Extensive clinical experience with DMSA and DMPS as antidotes and prophylactics for mercury, lead, arsenic and other heavy metals has been

reported in Soviet and mainland Chinese literature (10,11,12,13). In the Soviet Union, DMPS has been for many years an official drug called Unithiol. Recently, there has been a great deal of interest in both of these water soluble chemical analogs of dimercaprol in the United States and abroad (14,15,16,17,18). This has resulted in the confirmation and extension (19,20,21,22) of earlier reports dealing with both the basic and clinical investigations of DMSA and DMPS.

Although dimercaprol is a name relatively easily identified in the field of therapeutics, the compound is known most commonly in other areas as British Anti-Lewisite. It seemed reasonable to expect that a true analog agonist might also have Anti-Lewisite Activity.

The present experiments clearly show that either DMSA or DMPS will protect rabbits against the lethal systemic effects of subcutaneously administered lewisite (Table 1 and 2). Therefore, DMSA and DMPS can be considered to be not only analogous in chemical structure but also in anti-lewisite activity. In addition, DMSA and DMPS are effective when given by mouth; a route not recommended for BAL administration.

The dose schedule for administering DMSA and DMPS was based on a three day regimen recommended in the literature for the use of these metal binding agents. Subsequent studies (Aposhian, unpublished) have demonstrated that as little as one dose of 40 μmols/kg of either drug given im one minute after lewisite will result in the survival of 4 out of 6 rabbits. In addition when DMSA therapy is delayed until 90 min after lewisite, 6 of 6 rabbits, survived. The purpose of these studies was to determine whether DMSA or DMPS have anti-lewisite activity. No attempt has been made to quantitate their relative efficacy against lewisite.

Not only are these analogs crystalline and readily water soluble, they are less toxic than BAL. The results of a number of different investigations in rodents have led to the conclusion that the acute toxicity of DMSA is less than that of DMPS which is much less than that of BAL (19.23.24.25).

The stability studies (Fig 1) were initiated for two reasons. Many investigators believe that DMSA and DMPS are unstable because of their dimercapto structure. Since solutions of these compounds were being used throughout the day, for example see Table 1 and 2, it has been considered necessary by a number of investigators (17,20) to prepare solutions immediately before use. The stability of solutions of these dimercapto compounds is somewhat surprising since mercapto compounds are usually thought to be readily oxidized.

In addition to many older reports in the Soviet and Chinese literature (10,12,26) dealing with DMPS and DMSA in human therapy, such use has been strengthened by recent papers containing data from clinical investigations. For example, DMSA has been used recently in the treatment of a 46 yr. old man who ingested 2000 mg of arsenic in a suicide attempt (27). Treatment with 300 mg of DMSA every 6 hrs po for 3 days caused an increase in the urinary excretion of arsenic and eventual recovery. DMSA increased the excretion of lead in the urine of smelter workers and was effective in treating the signs and symptoms of lead poisoning (28). The dimercapto compound was well tolerated and no signs of toxicity were evident. The usefulness of DMPS and other metal binding agents in the treatment of mercury intoxication resulting from the Iraqi mercury disaster has been documented recently (18). DMPS, as DIMAVAL, is now an approved drug in West Germany for the treatment of mercury poison. These two water soluble analogs of BAL, analogous in activity as well

as chemical structure, active when given by mouth and of low toxicity, warrant continued investigation as possible replacements for BAL.

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BIOLOGICAL CHELATION: 2,3-DIMERCAPTO-PROPANESULFONIC ACID AND MESO-DIMERCAPTOSUCCINIC ACID

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INTRODUCTION

In 1946, summaries of the results of experiments dealing with a new metal binding agent appeared in the biomedical literature. The agent became known as British Anti-Lewisite or BAL. In the U.S. it was given the generic name of dimercaprol. Its importance initially was its effectiveness in treating exposure to the arsenic-containing chemical warfare agent, lewisite. Within a short time, BAL was shown to be useful in the treatment of intoxication by arsenic, lead, mercury and a number of other heavy metals. It was considered to be the long-sought universal antidote for poisoning by one or more of the heavy metals.

In subsequent years due to the increasing clinical experience and to the continuing search for better therapeutic agents, other chelating agents have been introduced (1). Some of these metal-binding agents have replaced one or more of the uses of BAL in clinical medicine. For example D-penicillamine is used to increase the excretion of copper in Wilson's disease (2) and N-acetyl-DL-penicillamine to treat mercury intoxication (3). The exception has been in the treatment of arsenic poisoning. Since the late 1940s, BAL has remained the drug of choice in the U.S. for treating arsenic poisoning (1). BAL, however, is far from the ideal drug. Some of its limitations are listed in Table 1.

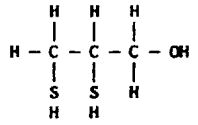
In the mid-1950s, the chelating properties of two new agents, the sodium salt of 2,3-dimercapto-1-propanesulfonic acid (DMPS) and meso-dimercaptosuccinic acid (DMSA) were reported (4, 5). These compounds are water soluble analogs of BAL whose structures are shown in Figure 1. The synthesis and some of the metal binding properties of DMPS were reported in 1956 by Petrunkin (4). DMPS is an official drug of the Soviet Union where it is known as Unithiol. The use of DMSA to increase the uptake of antimony during schistosomiasis therapy was reported by Friedheim *et al*, (5) in 1954. For the

TABLE I. SOME LIMITATIONS OF BRITISH ANTI-LEWISITE

1. High toxicity
2. Low therapeutic index
3. Unpleasant side effects
4. Limited water solubility
5. Instability in aqueous solution
6. Must be given by injection

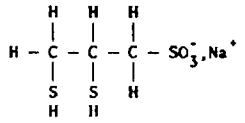
WATER SOLUBLE AND ORALLY ACTIVE

ANALOGS OF BRITISH ANTILEWISITE



BAL

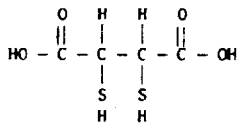
(BRITISH ANTILEWISITE)



DMPS

(2,3-DIMERCAPTO-1-PROPANE-SULFONIC ACID, Na SALT)

UNITHIOL, DIMAVAL



DMSA

(MESO-DIMERCAPTO SUCCINIC ACID)

SUCCIMER

FIG. 1. Water soluble and orally active analogs of British Anti-Lewisite.

next 20 years, many reports about the usefulness of these two dimercapto compounds appeared in the biomedical literature of the Soviet Union and mainland China. A few examples of these are cited (6–11). During this time, studies of these compounds by western investigators appear to be virtually nonexistent. (For example, it was not until 1975 that Friedheim and Corvi (12) reported the effectiveness of DMSA in treating mercury poisoning and it was not until 1976 that Gabard (13) reported the use of DMPS in mercury chelation therapy). The reasons for the paucity of earlier studies in the West may be that the synthesis of DMPS is very difficult and its export from the

Soviet to the West was prohibited. In the case of DMSA, although its synthesis is not as difficult, the main reason for a lack of investigative studies in the West appears to be that interest in and funds for chelation research were very limited. DMSA is called Succimer in the Soviet literature.

In about 1978, Heyl & Co., Berlin, succeeded in synthesizing and producing DMPS. This recent availability has encouraged investigators in West Germany, Norway and the U.S. to "rediscover" and study the drug with renewed interest (13–19). DMPS is marketed by Heyl & Co., as Dimaval. It is an approved drug in West Germany for the treatment of mercury intoxication. With the increasing need for safe and convenient chelating agents in clinical medicine, Dimaval should become an important addition to the physician's armamentarium.

The present paper summarizes experiments in this laboratory dealing with the experimental use of DMPS and DMSA in the treatment of poisonings of the following kinds: sodium arsenite in mice, lewisite in rabbits and cadmium chloride in mice. In addition, a summary of some of the important properties of DMPS and DMSA that has been retrieved from the Soviet literature will be discussed.

MATERIALS AND METHODS

Animals. Male mice of the Swiss CD1 strain (randombred Albino) were used in most of the experiments unless otherwise noted. At the time they were used in the experiments, they weighed approximately 25–30 g. Their source of purchase, their food and conditions for maintaining them have been described previously (16, 20).

Chemicals. DMPS in the form of its Na salt was a gift of Heyl & Co., Berlin. Since each molecule of NaDMPS has a molecule of H_2O associated with it, a molecular weight of 228.2 was used in mol calculations. DMSA used for the rabbit studies was pharmaceutical grade and a gift of Johnson and Johnson. The source of the other compounds have been described elsewhere (16, 20).

Biological studies. The assay of agents that bind and/or mobilize heavy metals can be based on a number of different measurable responses. The basis of one type of assay is the prevention or reversal of the lethal or toxic effects of the particular heavy metal. A second assay is based on the increased excretion of the metal by the putative metal binding agent. There is, however, increasing evidence that supports still another mechanism. Namely, a metal binding agent sometimes forms an insoluble metabolically-inert complex with the metal. The complex, because of its insolubility, is not excreted from the body. It remains in the cell, metabolically-inert and non-toxic. Therefore, it is possible that some metal binding agent might be life saving without increasing excretion of the metal. This mechanism has been proposed to explain the effectiveness of N-acetyl-DL-penicillamine (21). For these reasons we chose, as

the basis of our initial assays in the present work, the prevention of the lethal action of $NaAsO_2$. Eventually a quantitative comparison will be made of these agents as to their influence on the excretion of ^{74}As.

The concentrations of the $NaAsO_2$ solutions were prepared so that a 25 g animal would receive 0.050 ml. To quantitate the relative effectiveness of a compound in protecting against the lethal effects of $NaAsO_2$, the influence of the administration, i.p., of that compound on the LD_{50} of $NaAsO_2$ was determined by injecting, s.c., various amounts of $NaAsO_2$ dissolved in 0.9% saline. Solutions of the mercapto compounds were prepared immediately before use in 0.9% saline, adjusted to pH 5.5 using NaOH and the concentration adjusted so that a 25 g mouse would receive 0.10 ml. Injections were made using a 0.25 ml glass syringe with a No. 26 needle of 1/2 inch length. For oral administration, curved 18 gauge oral feeding needles, purchased from Popper & Sons, New Hyde Park, N.Y., were used. BAL was dissolved in peanut oil unless otherwise stated.

Statistical analysis. When appropriate, experimental results were analyzed using quantal response methodology. A logistic regression model was used to fit the experimental data and parameters were estimated using the BMDP program package (22) on a CDC Cyber 175 digital computer. Median effective dose and corresponding 95% confidence intervals were estimated following Finney (23).

RESULTS AND DISCUSSION

DMPS or DMSA Protects Mice Against the Lethal Effects of Sodium Arsenite

Mice injected with 0.14 mmols $NaAsO_2$/kg (an approximate LD 100 dose) and saline, in lieu of mercapto compounds, did not survive (Table 2). The deaths occurred within 48 hr after arsenic administration. DMPS and DMSA are potent antidotes (Table 2) when either agent is given intraperitoneally immediately after $NaAsO_2$. However, two other well-known and clinically useful chelating agents, D-penicillamine and N-acetyl-DL-penicillamine, do not protect (Table 2) under these conditions. The results with these two sulfhydryl compounds are unexpected since there have been two reports of the usefulness of penicillamine in the therapy of arsenic poisoning of humans (24–26).

Neither DMPS nor DMSA need be given immediately after $NaAsO_2$. The administration of either one of the compounds can be delayed at least 2 hr and still be effective (Table 3).

Of greater importance for any therapeutic or prophylatic potential is that DMPS or DMSA is effective even when given orally 15 min prior to the administration of the arsenic compound (Table 4). Under the present experimental conditions, they are effective as oral prophylactics against arsenic intoxication.

TABLE 2. PROTECTION BY DMPS OR DMSA AGAINST THE LETHAL EFFECTS OF SODIUM ARSENITE (16)

Chelating agent† (mmoles kg) i.p.	Cumulative 21-day survival No. surviving/No. started	%
(Saline)*	0/48	0
0.80 DMPS	32/32	100
0.40 DMPS	12/12	100
0.25 DMPS	24/24	100
0.14 DMPS	21/24	87.5
0.07 DMPS	19/24	79
0.25 BAL	22/24	92
0.14 BAL	2/24	8
0.25 DMSA	24/24	100
0.14 DMSA	20/24	83
0.07 DMSA	16/24	67
0.80 D-Pen	0/12	0
0.25 D-Pen	0/12	0
0.80 N-Ac-DL-Pen	0/12	0
0.25 N-Ac-DL-Pen	0/12	0

*The $NaAsO_2$ (0.14 mmoles/kg) was injected s.c. in the right rear leg.

†The chelating agents were administered i.p. immediately after $NaAsO_2$.

In this and subsequent Tables, the data represent the combined results of a number of separate experiments. The data were combined to take advantage of the resulting larger number of animals for the calculation of median doses, the statistical evaluation of data and the more economical use of publication space. Thus, the reason for the number of animals in some groups differing from the number in other groups of the same Table is that very often the combined data are the result of from 2 to 4 separate experiments in which different numbers of animals were used in each experiment. Otherwise, the experiments were performed under identical conditions. None of the mercapto compounds listed in Table 2 are toxic at the doses used and under the conditions of the present experiments.

TABLE 3. EXPERIMENTAL THERAPY WITH DMPS OR DMSA CAN BE DELAYED AFTER ARSENIC POISONING (16)

Dithiol and time after $NaAsO_2$* was given	Cumulative 21-day survival No. surviving/No. started	%
(Saline)	0/20	0
0.25 DMPS		
at 60 min	16/19	84
at 90 min	18/19	95
at 120 min	17/20	85
0.25 DMSA		
at 60 min	15/19	79
at 90 min	19/20	95
at 120 min	11/20	55

*All animals received $NaAsO_2$ (0.14 mmoles/kg) s.c. in the right rear leg. DMPS and DMSA were given i.p. At the start of the experiment, when $NaAsO_2$ was given, there were 10 animals in each group. However, in 3 of the experimental groups, one animal died before DMPS or DMSA was administered. Therefore, those groups are listed with 19 instead of the 20 started.

TABLE 4. PROPHYLACTIC AND ORAL ACTIVITY OF DMPS OR DMSA (16)

Thiol compound (mmoles/kg) oral	Cumulative 21-day survival No. surviving/No. started	%
Saline	0/28	0
1.0 DMPS*	16/18	89
0.75 DMPS	8/10	80
0.50 DMPS	16/20	80
0.25 DMPS	17/20	85
0.12 DMPS	0/10	0
1.0 DMSA	8/8	100
0.50 DMSA	10/10	100
0.25 DMSA	8/10	80
0.12 DMSA	4/10	40

The $NaAsO_2$ (0.14 mmoles/kg) was administered s.c. in the right rear leg. DMPS or DMSA was given orally 15 min prior to the $NaAsO_2$.

*The survival of control animals receiving 1.0 mmoles of DMPS per kg and saline, instead of $NaAsO_2$, was 100%.

The experiments summarized in Tables 2 to 4 demonstrate the effectiveness of DMPS and DMSA in protecting mice against the lethal action of arsenic. There does not appear to be a great difference between the effectiveness of these two agents under the present conditions. However, it is clear that D-penicillamine and N-acetyl-DL-penicillamine are without beneficial properties against the lethal effects of arsenic under the conditions used in these experiments. Although to our knowledge, arsenic chelate stability constants have not been determined for DMPS or DMSA, such constants, as well as the influence of DMPS in stimulating arsenic excretion, would be valuable in designing and determining the most effective chelating agent for therapy of arsenic poisoning.

Meanwhile, the relative effectiveness of a number of metal binding agents, with particular emphasis on DMPS and DMSA, has been evaluated quantitatively by determining their activity in changing the LD_{50} of $NaAsO_2$ in mice. In addition, the therapeutic index of DMPS and DMSA has been determined.

DMPS or DMSA increases the LD_{50} of $NaAsO_2$

The LD_{50} of subcutaneously administered $NaAsO_2$ was found to be 0.132 and 0.127 mmol/kg in 2 separate experiments. When the data of the 2 experiments were combined and used to determine the LD_{50}, it was found to be 0.129 mmol/kg (Table 5). The curve is remarkably steep, having a slope of 40.76, if the proportion survival vs dose model is used. The animals that did not survive usually died within 3 days after injection.

TABLE 5. LD_{50} OF SODIUM ARSENITE IN THE MOUSE (20)

$NaAsO_2$ (mmol/kg, s.c.)	Exp. 1 Dead / Started	Exp. 2 Dead / Started	Summation Dead / Started
0.08	0/8	—	0 8
0.09	0/8	—	0 8
0.10	0/8	0/12	0, 20
0.11	0/8	—	0/8
0.12	1/8	2/12	3 20
0.13	3/8	7/12	10, 20
0.14	7/8	12/12	19, 10
0.16	—	12/12	12, 12
LD_{50} (mmol/kg)	0.1315	0.1274	0.1290
95% Confidence interval	(0.122,0.260)	(0.080,0.131)	(0.125,0.139)

One way of quantitating the activity of a drug in overcoming the toxicity of ... agent is to determine how much the LD_{50} of the toxic agent is increased by giving more of the potential therapeutic drug. That is, the toxicity of the toxic agent should decrease by giving the therapeutic agent. When 2 i.p. injections of DMPS (0.80 mmols DMPS/kg/injection) are given, one immediately following and the other 90 min after the $NaAsO_2$, the LD_{50} of $NaAsO_2$ is increased approximately 4.2-fold to 0.538 mmol/kg (Table 6). Under the same conditions, but using DMSA instead of DMPS, the LD_{50} of $NaAsO_2$ is increased about 4.4-fold to 0.573 mmol/kg (Table 6). The increase with DMSA is only about 5% more than when DMPS is given. Since the LD_{50} of $NaAsO_2$ plus DMPS falls within the confidence interval of the LD_{50} of $NaAsO_2$ plus DMSA, it appears that the effect of DMPS and DMSA on the LD_{50} of $NaAsO_2$ is essentially the same under these experimental conditions.

Determination of Therapeutic Index

It was also of interest to determine and compare the therapeutic index of DMPS and DMSA as a measure of their relative potency. The therapeutic index under these conditions was determined by dividing the LD_{50} of the dimercapto compound by its ED_{50}. The latter value is defined as the amount of dimercapto compound (mmol/kg) protecting 50% of the animals against the lethal effects of 0.15 mmol $NaAsO_2$/kg. The latter dose kills 100% of the animals in this laboratory.

The LD_{50} of DMPS, when given i.p., was found to be 5.22 mmols kg (Table 7). This value is comparable to the value of 5.57 mmols/kg obtained by Kostygov (9) and 5.02 mmols/kg, i.p., in rats, as reported recently by Planashne *et al.* (27). For DMSA, the LD_{50} is 13.58 mmols/kg (Table 8). It ...pares favorably with 12.1 mmols/kg, i.p., found in mice by Shih-Chun *et al.* (11) in Shanghai and Peking and 14.0 mmols/kg determined by Matsuda

TABLE 6. DIMERCAPTO-1-PROPANE SULFONATE OR MESO-DIMERCAPTOSUCCINIC ACID INCREASES THE LD_{50} OF SODIUM ARSENITE* (20)

$NaAsO_2$ (mmol/kg, s.c.)	DMPS No. Dead / No. Started	DMSA No. Dead / No. Started
0.35	0/12	2/24
0.40	5/24	8/24
0.45	0/12	8/36
0.46	2/12	—
0.50	8/24	5/24
0.55	13/24	11/36
0.60	18/24	15/36
0.65	—	10/12
0.70	23/24	33/36
0.75	—	12/12
LD_{50} (mmol/kg)	0.538	0.573
95% Confidence interval	(0.492, 0.590)	(0.443, 0.708)

*DMPS or DMSA, 0.80 mmol/kg, was given, i.p. immediately after and 90 min after $NaAsO_2$.

TABLE 7. LD_{50} OF DIMERCAPTOPROPANESULFONATE IN MICE (20)

DMPS (mmols/kg, i.p.)	Dead / Started
3.3	0/8
4.0	0/8
5.0	7/16
5.5	5/8
6.0	7/8
6.6	15/16
7.0	8/8
9.9	8/8
LD_{50} (mmols/kg)	5.22
95% Confidence interval	(4.35, 5.51)

(10) in Japan. An LD_{50} in excess of 16.5 mmols/kg has been reported by Friedheim and Corvi (12). It is not clear whether this latter higher value is due to a difference in the mouse strains used or is due to a higher purity of DMSA. When mice were given $NaAsO_2$ (0.15 mmol/kg) s.c. and 10 min later were treated, i.p., with different amounts of DMPS, the ED_{50} was found to be 0.066 mmol/kg (Table 9). The ED_{50} under these conditions for DMSA was 0.065 mmol/kg. The therapeutic index for DMPS or DMSA under these conditions

TABLE 8. LD_{50} OF MESO-DIMERCAPTOSUCCINIC ACID IN MICE (20)

DMSA (mmols/kg, i.p.)	Dead / Started
6.0	0/32
12.0	8/32
13.0	6/12
14.0	9/12
16.0	19/24
18.0	17/20
24.0	32/32
LD_{50} (mmols/kg)	13.58
95% Confidence interval	(11.36, 15.22)

TABLE 9. DETERMINATION OF THE ED_{50} AND THERAPEUTIC INDEX OF 2,3-DIMERCAPTO-1-PROPANE SULFONIC ACID, NaSALT, AND MESO-DIMERCAPTOSUCCINIC ACID WHEN GIVEN 10 OR 35 MIN AFTER 0.15 mmols $NaAsO_2$/kg (20)

Dimercapto agent	DMPS + 10 min	DMSA + 10 min	DMPS + 35 min	DMSA + 35 min
(mmol/kg, i.p.)	number surviving/number started			
0.010	—	0/24	—	0/12
0.015	0/36	—	3/36	—
0.030	1/36	5/24	7/36	1/30
0.040	—	6/24	—	—
0.045	6/24	—	8/24	—
0.050	—	10/24	—	—
0.060	6/24	13/24	18/24	5/38
0.0675	15/24	—	—	—
0.070	—	9/12	—	-
0.075	21/24	—	—	—
0.080	—	18/24	—	5/12
0.090	20/24	—	15/24	3/10
0.100	—	—	—	16/28
0.105	31/36	—	30/36	—
0.120	35/36	—	34/36	8/12
0.125	—	21/24	—	13/17
0.150	—	—	—	21/30
0.160	—	—	—	6/8
0.200	—	—	—	37/46
0.300	—	—	—	35/38
ED_{50} (mmol/kg)	0.066	0.065	0.061	0.119
Confidence interval	(0.059-0.072)	(0.040-0.086)	(0.048-0.072)	(0.071-0.164)
Therapeutic index	79	209	86	115

was 79 and 209, respectively. When the DMPS and DMSA was given 35 min after the $NaAsO_2$, the therapeutic index was found to be 86 and 115, respectively. As can be seen under these conditions, DMSA can be considered to be a more effective agent than DMPS in protecting mice against the lethal effects of $NaAsO_2$ under these conditions.

Other metal binding agents were also tested for their activity in protecting against the lethal effects of $NaAsO_2$. Neither D-pen nor N-Ac-DL-Pen changes the LD_{50} of $NaAsO_2$ signficantly at the 95% level of significance (Table 10). Other agents (data not shown) that were also found to be ineffective in this respect are the sodium salt of diethyldithiocarbanate, α-mercaptopropionylglycine, DL-N-acetylhomocysteinethiolactone, and monomercaptosuccinic acid.

TABLE 10. NEITHER D-PENICILLAMINE NOR N-ACETYL-DL-PENICILLAMINE INCREASED THE LD_{50} OF SODIUM ARSENITE (20)

	none	D-Pen*	N-Ac-DL-Pen*
$NaAsO_2$ (mmols/kg, s.c.)	Dead / Started	Dead / Started	Dead / Started
0.10	0/12	0/8	0/8
0.12	2/12	5/8	1/8
0.13	7/12	7/8	5/8
0.14	12/12	8/8	4/8
0.16	12/12	8/8	8/8
0.20	—	8/8	8/8
LD_{50} (mmol kg)	0.127	0.119	0.133
95% Confidence interval	(0.080-0.131)	(0.078-0.191)	(0.054-0.142)

*D-pen or N-Ac-DL-pen (0.80 mmols/kg) was given, i.p., immediately following and at 90 min after the metal binding agent.

DMPS and DMSA Have Anti-Lewisite Activity

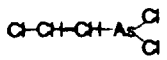
The name British Anti-Lewisite is ingrained very firmly in the mind of most biomedical investigators and physicians. One of the reasons for this is that most medical students and biomedical graduate students are told, in class, at one time or another, of the rational discovery and development of this metal chelating agent as an antidote for lewisite. This almost necessitates that any compound proposed as a replacement of BAL be shown to have anti-lewisite activity. Thus, we have tested DMPS and DMSA for their activity in protecting against the lethal effects of lewisite. The experiments were begun last March in collaboration with Drs. Brennie Hackley, Millard Mershon and Mr. Floyd Brinkley at the BioMed Laboratory at Aberdeen.

Lewisite is an arsenic containing CW agents. It is an oily liquid at 20°. It causes blisters, tissue destruction and blood vessel injury. Systemic poisoning

leading to death is possible. As a CW agent, it is considered to be a moderately delayed casualty agent. Its chemical formula is shown in Figure 2.

Since the introduction of British Anti-Lewisite at the beginning of World War II, all of the therapy of prophylaxis of lewisite has been aimed at chelating the arsenic in the molecule and making it biologically unavailable. This was the basis of Sir Rudolph Peters' search for British Anti-Lewisite.

In the present experiments, the rabbit is anesthetized and then shaved. Using a microliter syringe, lewisite (29.7 μmols/kg) is injected s.c. The volume of lewisite injected usually amounted to between 7 and 11 μl, depending on the weight of the animal. Table II shows that either DMSA or DMPS will protect rabbits against the lethal effects of lewisite. Using lewisite alone, none of the 6 animals survived. If the animals received 75 μmols DMSA/kg at the times indicated, all of the animals survived. DMSA protects against the lethal systemic effects of lewisite. In the second experiment, only 1 of 6 animals receiving lewisite survived; 66% survived when receiving the 75 μmols DMPS/kg regimen; and 50% of those receiving BAL survived. We do not wish to imply that the effectiveness of these agents against lewisite is in the order of DMSA > DMPS > BAL. More data are needed before relative effectiveness can be stated.



LEWISITE

(2-CHLOROETHENYL) - ARSONOUS DICHLORIDE

FIG. 2. Chemical formula for lewisite.

TABLE II. DMSA OR DMPS WILL PROTECT RABBITS AGAINST LETHAL EFFECTS OF LEWISITE

Expt.	μmols/kg*	7-day survival survival/start	%
I	LEW† —	0/6	0
	LEW + 75 DMSA	6/6	100
II	LEW + —	1/6	17
	LEW + 75 DMPS	4/6	66
	LEW + 75 BAL	3/6	50

*All agents were given s.c. except BAL, which was given i.m. The stated amount of dimercapto compounds were given at +1', +90', +180', +360' after lewisite and a.m. and p.m. on day 2 and 3.

†29.7 μmols lewisite/kg. These experiments were performed in collaboration with Drs. B. Hackley, M. Mershon and Mr. F. Brinkley.

The data, however, clearly show that DMSA or DMPS will protect rabbits against the lethal effects of lewisite. In this respect the compounds can be said to have Anti-Lewisite activity.

Treatment of Cadmium Toxicity

Cadmium is virtually ubiquitous. It is deposited and accumulates in most body tissues. It is found in all environmental compartments (air, soil, food and water). The study of cadmium biology has been stimulated by the debilitating osteoporosis of Itai-Itai disease in Japanese adults and the awareness that the increased use of cadmium in industrial and agricultural processes has greatly increased the prevalence of cadmium in the environment (28–30). Along with this, there has been an increased incidence of both acute and chronic cases of clinically identifiable cadmiosis (31). In the U.S., although the cadmium content of the human fetus is about 1 μg, the body burden increases approximately 30,000-fold (to about 30 mg) by age 50 years (32).

In experimental acute cadmium poisoning, DTPA or EDTA are marginally effective (30, 33–37) and dimercaptopropanol is contraindicated (37–39). It is astonishing that no accepted dependable effective drugs have become available for treatment of cadmium intoxication, especially since the biological effects of cadmium have been studied intensively during the last 20 years. Thus a number of research groups have been involved recently in a search for an agent to treat cadmium intoxication (18, 30, 41).

I would like to present in a very brief manner some of the unpublished results of my laboratory group as to attempts to find a therapeutically useful cadmium binding agent. DMPS protects mice against the lethal action of cadmium chloride (Tables 12, 13 and 14). Multiple ligand therapy, however, involving $CdCl_2$, DMPS and EDTA is ineffective. These observations (Tables 13 and 14) confirm those of Planas-Bohne (41) and to some extent those of Jones *et al.* (18).

In Table 14 are summarized the results with penicillamine and its analogs. Under the conditions of these experiments neither penicillamine nor any of its analogs so tested were active in protecting against cadmium lethality. Recent work by Yoshida *et al.* (40) with peptide fragments of mouse metallothionein is encouraging and should be extended to test peptide analogs of metallothionein fragments. Our own results are only a beginning and all studies with cadmium lethality and its therapy should be followed by kidney function tests.

Clinical Effectiveness in Man

Obviously the results of experiments summarized here and elsewhere warrant the continued investigation of these metal binding agents and their

TABLE 12. DMPS PROTECTS MICE AGAINST LETHAL ACTION OF CADMIUM CHLORIDE BUT MULTIPLE LIGAND THERAPY IS INEFFECTIVE

Group	$CdCl_2$ (mmol/kg) (i.p.)		Chelator(s) (mmol/kg) (i.m.)	Cumulative 28-day survival No. surviving/No. started	%
I	0.06	+	(saline)	13/56	23
II	0.06	+	1.0 DMPS	19/20	95
III	0.06	+	0.4 DMPS	12/20	60
IV	0.06	+	0.2 DMPS	6/30	20
V	0.06	+	0.50 $CaNa_2EDTA$	12/18	67
VI	0.06	+	0.10 $CaNa_2EDTA$	8/20	40
VII	0.06	+	0.05 $CaNa_2EDTA$	4/18	22
VIII	0.06	+	1.0 DMPS & 0.50 $CaNa_2EDTA$	10/10	100
IX	0.06	+	0.40 DMPS & 0.10 $CaNa_2EDTA$	2/12	17
X	0.06	+	0.20 DMPS & 0.10 $CaNa_2EDTA$	3/12	25
XI	0.06	+	0.20 DMPS & 0.05 $CaNa_2EDTA$	3/10	30
XII	(saline)	+	1.00 DMPS	9/10	90

*The i.m. injections were given 60 min after the i.p. injection of Cd.

†The one animal that did not survive in this group died on Day 21. Death appeared to be the result of fighting.

‡When saline was given i.p. instead of $CdCl_2$ and any of the following were given i.m. (mmol/kg) the survival was 100%: DMPS (0.80) or (0.20); $CaNa_2EDTA$ (0.50) or (0.05); DMPS (1.0) & $CaNa_2EDTA$ (0.5); DMPS (0.20) & $CaNa_2EDTA$ (0.05).

TABLE 13. DMPS GIVEN ORALLY INCREASES THE SURVIVAL OF MICE RECEIVING CADMIUM CHLORIDE (0.06 mmol/kg) I.P.

Group	Time after $CdCl_2$ that DMPS (1.0 mmol/kg)* was given orally (min)	Cumulative 28-day survival No. surviving/No. started	%
I	—†, —,	1/16	6
II	+10, + 90,	10/16	62
III	+10, + 90,	13/15	87
IV	—, + 90,	10/16	62
	The following groups are controls and received saline in place of $CdCl_2$		
V	+10, —,	14/16	88
VI	+10, + 90,	16/16	100
VII	—, + 90,	16/16	100

*The amount of DMPS given at each time.

†If DMPS was not administered, saline was given in its place.

TABLE 14. NEITHER PENICILLAMINE NOR ITS ANALOGS PROTECT MICE FROM THE LETHAL EFFECTS OF CADMIUM

Group	$CdCl_2$ (i.p.) (mmol/kg)	Thiol compound (oral) (mmol/kg)*	Min after $CdCl_2$ that thiol cmpd.	Cumulative 28-day survival	%
I	0.06	+ (saline)	—, —, —	2/32	6
II	0.06	+ 1.0 N-Ac-Pen	+10, + 90, + 180	1/8	13
III	0.06	+ 1.0 N-Ac-Pen	+10, + —, + —	0/8	0
IV	(saline)	+ 1.0 N-Ac-Pen	+10, + 90, + 180	8/8	100
V	0.06	+ 1.0 D-Pen	+10, + 90, + 180	15/16	94
VI	0.06	+ 1.0 D-Pen	+10, + —, + —	0/8	0
VII	0.06	+ 1.0 D-Pen	+10, + 90, + —	1/8	12
VIII	(saline)	+ 1.0 D-Pen	+10, + —, + —	8/8	100
IX	(saline)	+ 1.0 D-Pen	+10, + 90, + —	7/8	88
X	(saline)	+ 1.0 D-Pen	+10, + 90, + 180	7/8	88
XI	0.06	+ 1.5 N-Ac-thiolisoleuc	+10, + 90, + 180	0/8	0
XII	0.06	+ 1.0 N-Ac-thiolisoleuc	+10, + 90, + 180	3/16	19
XIII	(saline)	+ 1.5 N-Ac-thiolisoleuc	+10, + 90, + 180	6/8	75
XIV	0.06	+ 1.0 N-Ac-thiolisoleuc	+10, + —, + —	6/8	75
XV	(saline)	+ 1.0 ß-thiolisoleuc	+10, + —, + —	3/8	38
XVI	(saline)	+ 1.0 ß-thiolisoleuc	+10, + —, + —	9/9	100

*Amount given at each stipulated time.
†These results are the sum of 4 separate experiments.

clinical use in the treatment of heavy metal poisoning. There have been recent reports that confirm their effectiveness in human therapy. DMSA was found to be useful in the treatment of a 46 year-old man who ingested 2000 mg of arsenic in a suicide attempt (42). Treatment with 300 mg DMSA every 6 hr p.o. for 3 days caused an increase in the urinary excretion of arsenic with eventual recovery. DMPS has also been effective in human arsenic poisoning (Wager, personal communication). Friedheim *et al,* (43) in an extension of experiments with experimental animals have reported the effectiveness of DMSA in treating lead poisoning and that it increases the urinary excretion of lead in smelter workers. DMSA was well tolerated and no signs of toxicity were evident. The usefulness of DMPS in the Iraqi mercury disaster has been

TABLE 15. COMPARISON AND SUMMARY OF SOME OF THE INFORMATION ABOUT AND PHARMACOLOGICAL PROPERTIES OF DMPS AND DMSA

2,3-Dimercaptopropane-1-sulfonate, Na salt (DMPS, unithiol, dimaval)	Meso-2,3-dimercaptosuccinic acid (DMSA, Succimer)
1. Synthesized in 1950-51 at the Ukranian Res. Inst. for Health-Chemistry by Petrunkin. Published in 1956 (4).	1. Friedheim, 1954, used Sb-DMSA to increase Sb uptake in schistosomiasis therapy (5). (Intensively studied by mainland Chinese, 1959, for therapy of occupational metal poisoning (11). Primary Soviet investigator since 1965 has been Okonishnikova (50).
2. Crystalline powder, readily soluble in water. Very stable during sterilization and long-term storage.	2. Crystalline powder. Must be brought to pH 5–5.5 before completely soluble in water. Stability during sterilization and long term storage unknown.
3. Low toxicity, well tolerated even for chronic use, but DMSA is less toxic (20).	3. Toxicity is about 2.5 times less than DMPS (20).
4. Major toxic effect of high dose is hypotension (6, 8).	4. Major toxic effect of high dose unknown at present.
5. Distributed in extracellular space, exclusively (14). Excretion is urinary and rapid (14). Metabolic involvement supposedly none.	5. Distribution in body compartments unknown at present.
6. Effective antidote for As, Hg, Sb, Ag, Au, Cu, Cr, Pb, Po, Co, (6, 7, 16, 20, 45–49)	6. Effective antidote for As, Pb, Hg, Zn (16, 20, 50–54)
7. Urinary excretion of Cu and Zn. Increase Fe, Co, Mn or Ni excretion, none or minimal (55).	7. Urinary excretion of Co, Fe, Mn, Cu, or Zn, none or minimal (43).
8. Increase bile flow.	8. Effect on bile flow unknown.
9. Therapeutic dose about 250 mg for 70 kg man.	9. Therapeutic dose from 0.5 to 2 g for 70 kg man.
10. Can be given by mouth, s.c., i.p., i.m., i.v. Only 30–40% of oral dose absorbed from g.i. tract.	10. Can be given by mouth, s.c., i.p., i.m., i.v. Indications of oral dose being completely absorbed from g.i. tract.

documented recently (44). In fact DMPS, as DIMAVAL, is an approved drug in West Germany for the treatment of mercury poisoning.

There are many reports in the Soviet literature dealing with DMPS and DMSA both in experimental conditions or for human therapy. Some of them are cited in the summary of the properties of these two very important metal binding agents listed in Table 15. Obviously, these two water soluble analogs of BAL that are advantageous as to overall effectiveness and low toxicity can be expected to replace virtually all the therapeutic uses of British Anti-Lewisite.

SUMMARY

Water soluble analogs of British Anti-Lewisite that are active orally and less toxic than BAL are now available. These agents are 2,3-dimercapto-1-propanesulfonic acid and meso-dimercaptosuccinic acid. Evidence for their effectiveness in preventing the lethal effects of sodium arsenite in mice and lewisite in rabbits is presented. These analogs can be expected to replace BAL in the treatment of heavy metal poisoning.

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